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Original article submitted November 13, 1959

In the preceding work (Sung, 1960) it was established that when the T-1 variant of Aspergillus niger obtained through the effect of ultraviolet rays is cultured under surface conditions without changing the medium, it is characterized by an increase in the amount of phosphorus in the cells as compared with the original strain, 6/5. Upon further study, it was found that increased phosphorus content could be observed in all fractions of phosphorus compounds examined.

It was of interest to examine the phosphorus content of conidia and of mycelium of different ages in order to determine the significance of this change and the dynamics of distribution of phosphorus in the mycelium.

In addition to the usual cultivation without replacement, cultivation in which the medium was replaced was also conducted in the same manner as is done during the study of acid formation.

METHODS

Cultivation of Conidia and Production of an Acetone Preparation

In order to harvest substantial amounts of conidia, A. niger conidia of the original 6/5 culture and of variant T-1 were plated on Petri dishes of wort agar containing 1.5% table salt. Cultivation was carried out in an incubator at 32° for five days. The conidia were separated from the surface of the mycelium by suctioning with a pump and were collected in a flask of water. The conidial suspension was then filtered through bolting silk to remove conidiophores, and the conidia were washed repeatedly with distilled water so that the filtrate would contain no colored substances. After this, the conidia were suspended in anhydrous acetone chilled to 0° and were separated out of it by centrifugation. Acetone washing was carried out five times to the complete extraction of pigments.

The experimental methods have been given in detail in an earlier published communication (Sung, 1960). Inasmuch as there were no changes in methodology, only the results of the experimental work are given in the present communication.

EXPERIMENTAL PART

The Content of Phosphorus Compounds in Conidia

Table 1 illustrates the character of distribution of phosphorus compounds in A. niger conidia of the original 6/5 strain and of its T-1 variant obtained by means of uv treatment.

From the data presented in Table 1, it follows that certain differences exist in the distribution of phosphorus compounds in the conidia of both strains. First, the conidia of variant T-1 contain more total phos-

Table 1. Phosphorus Compounds in the Conidia of the Original 6/5 Strain of Aspergillus niger and of Its T-1 Variant

Forms of phosphorus	Original strain 6/5		Variant T-1		Ratio variant: :original
	γ/200 mg	% total P	γ/200 mg	% total P	
Total phosphorus	1948.0		2048.0		1.05
Acid-soluble phosphorus	322.0	16.4	380.0	18.5	1.18
Inorganic	123.8	6.4	163.7	7.9	1.32
Labile	12.3	0.6	8.6	0.4	0.70
Stable	185.9	9.4	207.7	10.1	1.12
Phospholipids	112.0	5.8	155.0	7.5	1.45
Phosphorus of total nucleic acids	756.3	38.8	812.5	39.6	1.07
Nonnucleotide phosphorus (polyphosphate)	776.2	39.8	695.0	33.8	0.90
Residue after $HClO_4$ extraction (phosphoproteins)	74.0	3.8	6.1	0.3	0.08

Table 2. Phosphorus Compounds in the Mycelium of Strain T-1 When Cultured by the Nonreplacement Method (amount of phosphorus in γ per 200 mg of dry weight of mycelium)

Form of phosphorus	Cultivation time							
	2 days		3 days		4 days		5 days	
	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P
Total phosphorus	1446	100	1028	100	888	100	714	100
Acid-soluble phosphorus	378.3	26.2	329.2	32.0	314.8	35.5	263.5	36.9
Inorganic	62.2	4.3	121.1	11.8	98.2	11.0	48.1	6.7
Labile	149.3	10.3	184.4	17.9	143.1	16.1	160.9	22.5
Stable	166.8	11.5	23.7	2.3	73.5	8.7	54.5	7.6
Phospholipids	48.7	3.4	39.0	3.8	37.1	4.1	28.7	4.0
Nucleic acids	554.5	38.4	273.5	26.6	78.5	20.0	110.6	15.5
Nonnucleotide phosphorus (polyphosphate)	229.5	15.9	228.4	22.2	210.7	23.8	203.9	28.6
Residue after $HClO_4$ extraction (phosphoproteins)	225.4	15.6	158.9	15.5	147.2	16.6	106.4	14.9

Table 3. Phosphorus Compounds in the Mycelium of Strain 6/5 When Cultured by the Nonreplacement Method (amount of phosphorus in γ per 200 mg of dry weight of mycelium)

Form of phosphorus	Cultivation time							
	2 days		3 days		4 days		5 days	
	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P
Total phosphorus	1150	100	764	100	610	100	324	100
Acid-soluble phosphorus	332.0	28.9	261.3	34.2	209.7	34.4	101.7	31.4
Inorganic	49.9	4.3	97.9	12.8	50.1	8.2	26.0	8.6
Labile	133.4	11.6	157.1	20.5	119.7	19.6	59.8	18.5
Stable	148.7	12.9	6.3	0.8	39.9	6.5	15.9	4.9
Phospholipids	35.7	3.1	29.2	3.8	27.8	4.5	25.5	7.7
Nucleic acids	399.6	34.8	170.7	22.4	124.4	20.4	70.6	21.8
Nonnucleotide phosphorus (polyphosphate	216.5	18.8	160.5	21.0	142.1	23.3	68.0	21.0
Residue after $HClO_4$ extraction (phosphoproteins)	157.6	13.7	126.2	16.5	107.1	17.5	57.7	17.8

phorus (10.24 γ /mg of dry weight of the conidia) than do those of the original strain 6/5 (9.74 γ /mg of dry weight of the conidia). Included in this, in variant T-1, 18.5% of total phosphorus was in the acid-soluble fraction and 73.7% in the acid-insoluble fraction, while in the original strain—it was 16.4% and 82.4%, respectively.

It was striking that only a very insignificant part of the total phosphorus was in the labile form (0.4%—variant T-1, 0.6%—original strain).

As seen from the table, the ratios of phospholipids and phosphoproteins in the variant and in the original strain differed greatly. In variant T-1, the phospholipid content was about 45%, i.e., higher than in the original strain, while the phosphoprotein content was considerably less than in 6/5. From the data given, it can be seen that the amount of phosphorus forms in these two strains differ from one another in absolute values, but the relative distribution of the amount of individual fractions has a common pattern.

The Content of Individual Phosphorus Fractions in the Medium of a Growing Culture when Grown Without Exchange of Medium

Results of the investigations are presented in Tables 2 and 3. As seen from the tables, the maximal phosphorus content in the mycelium was observed at the early stages of development (on the second day). Then, the amount of phosphorus decreased.

The time of maximal phosphorus content coincided with intensive mycelial growth. Toward the end of the period of intensive mycelial growth, the amount of phosphorus decreased considerably.

Acid-soluble phosphorus constituted the main portion of total phosphorus. With age, an increase in the per cent of acid-soluble phosphorus compounds occurred, including labile phosphorus as well.

An entirely different picture was observed with respect to the distribution of the amount of nucleic acids. As the age of the culture increased, the amount of nucleic acid phosphorus dropped if expressed in per cent of total phosphorus; thus, for example, whereas in variant T-1 nucleic acid phosphorus constituted 38.4% of total phosphorus on the second day, on the fifth day it was 15.5%. In the initial 6/5 strain it was 34.8% and 21.8%, respectively.

Phospholipids were found in relatively smaller amounts and constituted approximately 3–4% in variant T-1 and 3–7% in the original 6/5 strain.

It should be noted that the data given in Tables 2 and 3 on the content of nonnucleic phosphorus represent the difference between total phosphorus extracted by 0.5 N $HClO_4$ at 98° and the phosphorus of nucleic acids. Judging by data in the literature, this excess phos-

Table 4. Ratio of Different Phosphorus Compounds in Mycelium During the Replacement Method of Cultivation $\left(\frac{T-1}{6/5}\right)$

Form of phosphorus	Cultivation time			
	2 days	3 days	4 days	5 days
Total phosphorus	1.26	1.35	1.45	2.20
Acid-soluble phosphorus	1.14	1.26	1.49	2.58
Inorganic	1.24	1.25	1.96	1.84
Labile	1.13	1.18	1.20	2.67
Stable	1.10	4.00	1.85	3.38
Phospholipids	1.33	1.25	1.36	1.07
Nucleic acids	1.38	1.54	1.43	1.54
Nonnucleotide phosphorus (polyphosphate)	1.06	1.42	1.50	3.0
Residue after $HClO_4$ extraction (phosphoproteins)	1.45	1.25	1.37	1.79

Table 5. Distribution and Transformation of Phosphorus Compounds in the Mycelium of a Growing Culture of Aspergillus niger, Variant T-1, During the Replacement Method of Cultivation

Form of phosphorus	Without replacement of solution						After replacement of solution			
	Cultivation time						Time of cultivation after adding sugar solution			
	2 days		4 days		6 days		4 days		6 days	
	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P
Total phosphorus	1790.0	100	1104.0	100	694.0	100	1082.0	100	932.0	100
Acid-soluble phosphorus	595.0	33.1	435.0	39.4	302.5	43.5	450.0	41.5	475.2	51.0
Inorganic	218.5	12.2	140.1	12.7	117.4	16.9	177.4	16.4	177.5	19.6
Labile	162.7	9.1	197.4	17.9	126.6	18.2	150.1	13.9	110.0	11.8
Stable	213.8	11.9	97.5	8.8	58.5	8.4	122.5	11.3	187.7	20.1
Phospholipids	46.7	2.6	33.2	3.0	21.5	3.1	143.1	13.2	173.2	18.6
Phosphorus of total nucleic acids	658.0	37.4	224.8	20.4	96.5	13.9	189.6	17.5	128.2	13.7
Nonnucleotide phosphorus polyphosphate	253.3	14.1	281.3	25.4	155.4	22.4	241.9	22.3	52.1	5.6
Residue after $HClO_4$ extraction (phosphoproteins)	241.2	13.5	119.8	10.8	114.2	16.4	69.4	6.4	103.9	11.2

phorus is polyphosphate. The amount of this phosphorus fraction also rose during the course of the entire fermentation. The phosphoprotein content changed insignificantly during the course of the entire period of growth.

Going on to a comparison of the results given above which were obtained in the nonreplacement cycle of development of variant T-1 and the original 6/5 strain, the similar pattern in the dynamics of phosphorus distribution during growth must be pointed out.

This fact suggests that these two strains have high acid-forming activity and therefore, at the time of vigorous acid production, they have a similar type of distribution of phosphorus forms.

However, more accumulation of phosphorus occurred in the experimentally obtained T-1 variant during the same growth time than in 6/5 (Table 4). Therefore, we propose that variant T-1 is characterized by substantial shifts in phosphorus metabolism. The experimentally obtained strain apparently has increased phosphorus metabolism activity at all stages of development, although biomass synthesis is inhibited in T-1 and it lags behind strain 6/5 in biomass increment during the entire period of development.

Content of Various Forms of Phosphorus When the Medium is Replaced

In Tables 5 and 6, analytical data are given on the investigation of growing cultures of A. niger 6/5 and variant T-1 when the medium is replaced.

As seen from these data, when a sugar solution was poured under the film, a noticeable change in the distribution of phosphorus compounds occurred in the cells of the films.

The decrease in total phosphorus of the mycelium in this case also was accompanied by an increase in acid-soluble phosphorus compounds, which represented the main portion of total phosphorus in both cultures throughout the duration of the replacement cycle.

Table 6. Distribution and Transformation of Phosphorus Compounds in the Mycelium of a Growing Culture of the Original 6/5 Aspergillus niger During the Replacement Method of Cultivation

Form of phosphorus	Without replacement of solution						After replacement of solution			
	Cultivation time						Time of cultivation after adding sugar solution			
	2 days		4 days		6 days		4 days		6 days	
	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P	γ/200 mg	% total P
Total phosphorus	1216.0	100	846.0	100	416.0	100	804.0	100	676.0	100
Acid-soluble phosphorus	438.6	36.0	344.0	40.6	178.3	42.9	338.6	42.1	336.8	49.8
Inorganic	122.7	10.1	108.3	12.8	69.6	16.7	135.1	16.8	147.4	21.8
Labile	150.7	12.4	164.2	19.4	68.7	16.5	105.2	13.1	71.8	10.6
Stable	164.0	13.5	71.1	8.4	41.6	9.7	97.3	12.1	117.6	17.4
Phospholipids	45.0	3.7	35.7	4.2	22.8	5.1	87.9	10.9	92.2	13.6
Phosphorus of total nucleic acids	402.4	33.0	178.5	21.1	82.6	19.8	123.4	15.4	91.7	13.5
Nonnucleotide phosphorus (polyphosphate)	199.8	17.9	181.3	21.4	105.4	25.4	156.7	19.5	72.3	10.7
Residue after $HClO_4$ extraction (phosphoproteins)	130.2	10.7	107.8	12.7	65.5	15.7	80.2	10.2	96.3	14.2

The fact established by us of the increase in amount of phospholipids during cultivation with replacement of medium is of interest. This phenomenon makes it possible to assume that, with the pouring of the sugar solution, the transformation of phosphorus consumed at the time of growth on the first medium takes place in the fungal cells along with the synthesis of acids.

DISCUSSION OF RESULTS

When comparing the data obtained, it follows that there are no essential differences in the dynamics and distribution of phosphorus compounds in these two strains of A. niger. However, we obtained large divergences in the quantitative content of phosphorus compounds in the strains tested both in the replacement cycle and during cultivation by the nonreplacement method.

Pomoshchnikova (1956), who subjected yeasts to the action of x rays, notes that an increase in the amount of phosphorus compounds is observed in the next generation of irradiated yeast cells. In the opinion of the author, the reason for this accumulation of phosphorus is a delay in its normal utilization by the irradiated cell in which synthetic processes are retarded. The same thing is possibly observed in the case of uv variants of A. niger as well.

Foster (1949) suggests that the small amount of phosphates in fungal conidia is explained by the slight accumulation of energy-rich phosphorus esters.

The results which we obtained in the present work with strain 6/5 of A. niger and with variant T-1 showed that considerable accumulation of phosphates takes place in the conidia of these strains. This correlates with the results which were obtained by Krishnan, et al. (1954), and in Belozerskii's laboratory (1957). On the other hand, it should be noted that the major amount of phosphorus going into the conidia is accumulated in the acid-insoluble fraction, and only a very small part of it in the labile form is found in the acid-soluble fraction.

As far as the redistribution of phosphorus compounds in the mycelium after the replacement of medium by sugar solution is concerned, the picture of the dynamics of phosphorus differs markedly from that which is observed during nonreplacement cultivation. It is sufficient to point out that when the mycelium does not obtain phosphorus from without, a redistribution of the phosphorus compounds in the internal resources of the mycelium occurs. These data could confirm the hypothesis that the phosphorylation process is involved in the production of citric acid. It is well known that a theory existed in the literature that phosphorylation does not participate in citric acid synthesis because the acid is produced on sugar solution containing no phosphorus.

In this case, it was overlooked that there is a possibility of secondary redistribution of phosphorus compounds accumulated in the mycelium during growth in phosphate-containing solution.

In conclusion, I should like to take this opportunity to express deep appreciation to Prof. A. A. Imshenetskii and E. L. Ruban for advice and critical comments during the performance of the work.

SUMMARY

1. The conidia of the T-1 strain differ greatly from the original 6/5 strain with regard to the absolute amount of phosphorus in various fractions of conidia.

2. The relative phosphorus content of various fractions is similar in both strains.

3. A high proportion of phosphorus in the conidia of these strains is represented by the phosphorus of nucleic acids and by acid-insoluble polyphosphates.

4. The mycelium of the T-1 strain is richer in all fractions of phosphorus compounds tested than the 6/5 mycelium at all developmental stages.

5. The relative distribution of individual phosphorus forms in T-1 and 6/5 is similar both in the replacement and nonreplacement methods of cultivation.

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A NEW SPECIES OF THE ACTINOMYCES AURANTIACUS GROUP

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In 1958, during the screening for antagonist actinomycetes from brown soil taken from under cotton plants in the neighborhood of Peking (CPR), actinomycete strain No. 1077 was isolated, which differs in its outward and physiological properties from those earlier described in the literature. This strain is characterized by purplish-orange color and by verticillate structure of the sporophores.

A verticillate actinomycete was first described by Waksman (1916) and was named Actinomyces reticuli by him. In 1919, Waksman and coworkers described a new species of verticillate actinomycetes (Actinomyces reticulus-ruber). Kriss (1938) described a verticillate actinomycete under the name of Actinomyces verticillatus. In 1941, Krasil'nikov described an actinomycete which he named Actinomyces circulatus. In recent years, Shinobu (1955, 1956), Nakazawa (1955), Yamaguchi (1954), Shinobu (1958), Sugawara (1956), and Yen Sun-ch'u (1956) have described new species of the genus Actinomyces with verticillate branching which they named A. hiroshimensis, A. roseoverticillatus, A. albireticuli, A. hachijoensis, A. spiroverticillatus, A. caespitosus, and A. rubroverticillatus.

METHODS

In the study of morphological and physiological properties, media were employed which are described in Krasil'nikov's (1950) and Waksman's (1950) manuals and Nikitina's dissertation (1957).

The determination of antagonistic properties was carried out by the method of superimposing agar blocks of the culture under investigation on a freshly-prepared background of a test organism. For this, the experimental culture was grown on media optimal for antibiotic production (fish agar, CP-1Y* +0.5% soy meal) in Petri dishes for seven days at 27°.

The detection of antagonism toward phages was carried out by the method described in the work of Krasil'nikov and Kofanova (1957). Nine strains of actinophages and four strains of bacteriophages were taken as tests.

The assimilation of various carbon sources was determined by the Pridham and Gottlieb method (1948).

EXPERIMENTAL RESULTS

Morphology

The culture grows well on many agar media: CpI, CpIII, CpIV, glucose-asparagine, starch-ammonia, potato, fish, and MPA. However, whorls form only on the following media: CpI, CpIV, glucose-asparagine, starch-ammonia, and potato. The whorls are primary and secondary. The sporophores are long, straight, or slightly curved. An incomplete half-turn or full-turn coil is formed on the ends of the sporophores (Fig. 1). The spores are elongated (Fig. 2).

Cultural Characteristics

On medium CpI—the culture grows well, the colonies are quite bright, of a dark orange color (p-6 on Bondartsev's scale, 1954) and frequently purplish-orange. The mycelium penetrates deep into the agar.

The medium is never colored. Border of the colony is fringed. Aerial mycelium is well developed, pinkish, velvety. The sporophores are verticillate; an incom-

*As in original—Publisher.

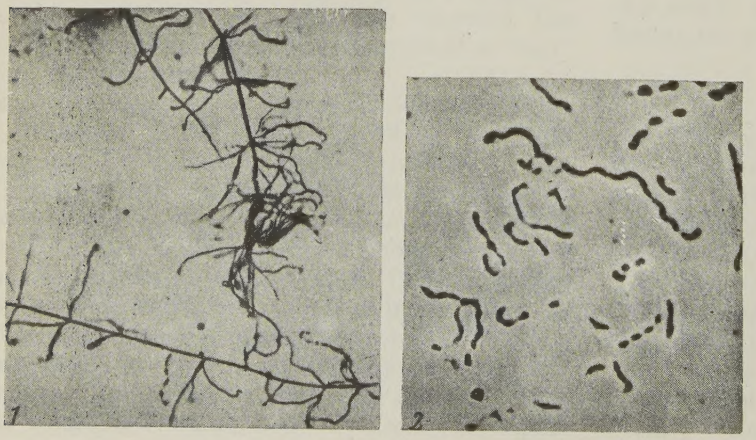

Fig. 1. Sporophores. Magnification 300×. Primary and secondary whorls. The sporophores have an incomplete coil on the end: a) on Czapek's medium with glucose (12th day of growth); b) on potato agar.
Fig. 2. Spores. Magnification 350×. Elongated, on Czapek's medium with glucose (7th day of growth).

Table 1. Antimicrobial Properties of Culture No. 1077

Media \ Test cultures	Staph. aureus	E. coli	Proteus vulgaris	Bact. prodigiosum	Ps. pyocyanea	Bac. mycoides	Bac. anthracoides	Bac. subtilis	Bac. idosus	Mycobact. B-5	Mycobact. citreum	Mycobact. luteum	Rh. trifolii (327)	Rh. melilоti (413)	Rh. leguminosarum (225a)	Rh. phaseoli (11)	Rh. viciae
Fish agar	8	0	0	±	0	6	10	10	5	8	8	6	0	0	0	0	0
CpIV with 1.5% soy meal	4	0	0	±	0	2	3	8	2	4	8	3	0	0	0	0	0

Note. Figures designate radius of the zone of absence of growth of the test organism in millimeters; ± designates a variable effect.

Table 2. Antifungal Properties of Culture No. 1077

Media \ Test cultures	Sacch. cerevisiae	Candida albicans	Debaryomyces sp.	Monilia sp.	Torulopsis sphaerica	Torulopsis kefyri	Torulopsis lactis	Sporobolomyces philippovi	Pen. chrysogenum	Asp. niger	Phomopsis sp.	Deuterophoma tracheiphila	Botrytis albi	Alternaria solani	Alternaria humicola	Helminthosporium sativum	Fus. vasinfectum	Fus. oxysporum	Fus. moniliforme	Fus. graminis	Piricularia oryzae
Fish agar	1	1,5	6	1	2	1	1	4	4	3	6	8	7	6	2	10	3	2	8	2	6
CpIV with 1.5% soy meal	0	0	3	0	0	0	0	2	2	1	4	6	7	6	1	6	3	0	4	1	3

Note. Designations the same as in Table 1.

Table 3. Conditions of the Production of Antimicrobial Substances by Culture No. 1077

Media \ Test cultures	Staph. aureus	E. coli	Proteus vulgaris	Bact. prodigiosum	Ps. pyocyanea	Bac. subtilis	Bac. anthracoides	Mycobact B-5	Mycobact. citreum	Sacch. cerevisiae	Candida albicans	Monilia sp.	Asp. niger	Helminthosporium sativum	Alternaria solani	Fus. vasinfectum	Fus. oxysporum	Fus. moniliforme
Fish medium	8	0	0	±	0	12	10	7	5	1	1	2	2	10	10	3	1	8
MPA (without glucose)	5	0	0	0	0	5	6	2	0	0	0	0	0	6	8	1	0	4
Potato	5	0	0	0	0	6	5	7	2	0	0	0	1	8	8	2	0	3
Corn	4	0	0	0	0	7	7	7	3	0	0	0	1	8	8	2	0	5
Soy	5	0	0	0	0	7	6	7	2	0	0	0	1	8	8	2	0	5
Czapek's	4	0	0	0	0	5	3	5	3	0	0	0	0	4	6	2	0	3

Note. Designations the same as in Table 1.

plete half-turn and less frequently a single-turn coil is formed at their end.

On medium CpIII—poor growth, rusty-orange colonies. Aerial mycelium absent or is produced in the form of a slight film consisting of single short aerial branches. The medium does not become colored.

On medium CpIV—good growth. The colonies are slightly folded. The border is fringed, bright orange in color. Mycelium penetrates deep into the agar. The medium does not become colored. Aerial mycelium is well developed, orange or pink, velvety.

On starch-ammonia agar—good growth, colonies orange-red or pale crimson, borders of colony fringed. Filaments of mycelium penetrate deep into the agar. The medium does not become colored. Aerial mycelium pink, velvety.

On potato agar—good growth, colonies folded, orange in color. Aerial mycelium well developed, pinkish in color, velvety. The medium does not become colored.

On wort agar—good growth, colonies folded, colorless at first, then acquire an orange-crimson color, mycelium penetrates deep into the agar. Aerial mycelium poorly developed. The medium does not become colored.

On medium with fish extract—good growth, colonies very folded, slightly orange at first, then bright orange or rusty orange. The medium does not become colored. Aerial mycelium well developed, pink, velvety. Whorls are rarely produced on this medium. Sporophores verticillate, predominantly straight, very infrequently with a half-turn coil on the end.

On meat-peptone agar—poor growth, colonies small, folded, bare, without aerial mycelium or it is present in the form of a slightly noticeable film. The culture is colorless at first, then it acquires a reddish-brown color. The medium does not become colored.

On carrot slices—no growth.

On potato slices—good growth. Colonies bright orange in color. Aerial mycelium well developed, pink.

Biochemical Properties

The culture decomposes starch vigorously; the hydrolysis zone reaches 5–7 mm on the fifth day. Does

Table 4. A Comparison of Culture No. 1077 with Actinomycetes Described in the Literature Which Have Verticillate Sporophores and with Actinomyces aurantiacus

Name	Sporophores	Spores	Colonies	Aerial mycelium	Production of pigment in medium	Antagonistic properties
Act. verticillatus (Kriss, 1936, 1938)	Whorls primary. Sporophores straight	Cylindrical and elongated	Colorless or slightly brownish	White at first, then dark gray or gray-green	Not produced	Not determined
Act. circulatus (Krassilnikov, 1941, Krasil'nikov, 1949)	Whorls primary. Sporophores spiral, arranged in whorls, spirals with 1–2 turns	Cylindrical and elongated	Colorless	Whitish	Not produced	Not determined
Act. reticuli (Waksman and Curtis, 1916)	Whorls primary and secondary. Sporophores long, straight, spiral with 2–3 turns	Spherical or slightly oval	Colorless or slightly brownish	Whitish with a yellowish tint	Brown substances secreted on protein media	No indication
Act. spiroverticillatus n. sp. (Shinobu, 1958)	Whorls usually primary, rarely secondary. Spirals usually have 1–2 coils, rarely—3. Loose coils	Spherical or oval	Colorless, light, brown-yellow-orange	Fine, white	Not produced on synthetic agar	Not determined
Act. reticulus-ruber (Waksman et Curtis, 1919).	Whorls primary and secondary. Sporophores spiral	Oval	Bright pink or red	Fluffy, white or pinkish-white	A brown substance is produced on protein media which diffuses into the substrate	Acts on Gram-negative and Gram-positive bacteria, Mycobacteria
Act. hachijoensis (Yamaguchi, 1954)	Whorls primary and secondary. Sporophores spiral	Cylindrical	Colorless	White at first; then pinkish-buff color	Not produced on synthetic agar	Acts strongly on yeasts
Act. caespitosus (Sugawara, 1956)	Whorls primary, fruiting branches straight	Oval	Colorless to pale yellow-brown	Fine, white with pale yellow, brown, or greenish-yellow tint	Pale yellow on synthetic agar	Not determined
Act. albireticuli sp. (Nakazawa, 1955).	Whorls primary and secondary. Sporophores nonspiral	Oval, spherical	Colorless	White	Not produced on synthetic agar	Does not act on Escherichia coli; acts on Gram-positive bacteria
Act. hiroshimensis (Shinobu, 1955)	Whorls primary and secondary. Sporophores nonspiral	Oval, spherical	Pink	Light, pinkish-white	Not produced on synthetic agar; pale brownish-orange with red tint produced on organic media	Does not act on Escherichia coli; acts on Gram-positive bacteria
Act. roseoverticillatus Shinobu, 1956).	Whorls primary and secondary. Sporophores not spiral	Spherical or oval	Light, pink, red	Fluffy, light, pink, white	Not produced on synthetic agar, while on organic media it is brown or brownish-red	Not determined
Act. rubroverticillatus (Yen Sun-ch'u, 1956)	Whorls primary and secondary. Sporophores straight, short	Cylindrical	Bright red	White	Pale yellow produced on synthetic agar	No indication
No. 1077 Act. aureoverticillatus n. sp.	Whorls primary and secondary. Sporophores form incomplete coil, arranged in whorls of 2–5 or more in each	Elongated	Orange-rust, purplish-orange. Borders fringed	Velvety, pink	Not produced on all media	Does not act on Escherichia coli; acts on yeasts and fungi, Gram-positive bacteria, mycobacteria
Act. aurantiacus Krassilnikov (Krasil'nikov, 1941)	Poor sporophore production. No whorls, principally straight or slightly coiled spirals	Spherical and oval	Bright orange	Absent or produced in the form of a slight film consisting of separate short aerial branches	Not produced on all media	Does not act on Escherichia coli; acts on Gram-positive bacteria

not peptonize milk or peptonizes it very slowly. A small layer of peptonized milk appears in the upper part of the test tube only on the 20–30th day. The strain does not produce hydrogen sulfide. Grows well on filter paper, but does not decompose it. Liquefies gelatin slowly. The pigment produced by the culture is extracted by alcohol, acetone, benzine, and chloroform but is not soluble in water. According to Kriss' system (1936), the pigment is more closely related to the lipoactinochromes.

Assimilation of Various Carbon Sources

According to the data of Krasil'nikov and coworkers, outwardly identical cultures of actinomycetes (comprising a single group) can easily be differentiated on the basis of their ability to assimilate different sources of carbon nutrients.

In Pridham and Gottlieb's medium, culture No. 1077 does not assimilate sucrose, lactose, rhamnose, raffinose, sodium acetate, or sodium oxalate. It assimilates arabinose, galactose, inositol, glucose, maltose, mannitol, and sodium citrate well.

Antimicrobial Properties

The culture being described has strongly pronounced antimicrobial properties. It produced antibiotic substances on various media—both complex organic and synthetic ones. It produced the largest amount of antibiotic substances on fish medium (Table 3). In Tables 1 and 2, data are given on the spectrum of antimicrobial activity, from which it is seen that culture No. 1077 strongly inhibits the growth of Gram-positive bacteria—staphylococci (Staphylococcus aureus), sporogenous bacteria (Bacillus mycoides, Bacillus mesentericus, Bacillus subtilis, Bacillus idosus) and also mycobacteria (Mycobacterium B-5, acid-resistant strain, Mycobacterium citreum, Mycobacterium luteum).

The culture does not affect Gram-negative bacteria; no inhibition of growth was noted in Escherichia coli, Bacterium proteus, Pseudomonas pyocyanea, Rhizobium (R. trifolii, R. meliloti, R. leguminosarum, R. phaseoli, R. viciae); a variable effect was noted when acting on Bacterium prodigiosum.

Strain No. 1077 actively inhibits the growth of yeasts and yeast-like organisms: Saccharomyces cerevisiae, Candida albicans, Debaryomyces sp., Monilia sp., Torulopsis sphaerica, Torulopsis kefyri, Torulopsis lactis, Sporobolomyces philippovi. It also inhibits the growth of the following fungi: Penicillium chrysogenum, Aspergillus niger, Phomopsis sp., Deuterophoma tracheiphila, Botrytis albi, Alternaria solani, Alternaria humicola, Helminthosporium sativum, Fusarium vasinfectum, Fusarium oxysporum, Fusarium graminis, Fusarium moniliforme, Piricularia oryzae.

The culture studied exhibited no inhibitory effect on the following bacteriophages and actinophages: A. olivaceus 8238, A. erytreus No. 3, A. streptomycini type III, A. levoris No. 9, A. globisporus 1117/1203, A. lavendulae 3613/1664, A. venezuelae 10712/8231 polyphage 105, A. gobitricuni 2335/3613, Staphylococcus albus, Staphylococcus sp., E. coli, Bacillus megaterium.

Culture 1077 was not sensitive to a single one of the actinophages tested. Culture No. 1077 breaks down plant tumors caused by Pseudomonas tumefaciens.

DIFFERENTIAL DIAGNOSIS

Actinomycete culture No. 1077 differs from both colorless and pigmented verticillate actinomycete species described earlier. It differs from the colorless ones (A. verticillatus, A. circulatus, A. reticulis, A. hachijoensis, A. caespitosus, A. albireticuli) by bright pigmentation of the colonies as well as by the pink or orange color of the aerial mycelium. Culture 1077 is more closely related to the pigmented verticillate actinomycetes which produce red-orange pigment (A. spiroverticillatus, A. rubroverticillatus, A. roseoverticillatus, A. reticulus-ruber, and others). However, upon more thorough comparison, it differs from these organisms as well. First, its sporophores always produce half-turn or single turn coils on the end, which is not observed in other species. In the species described in the literature, sporophores are either straight without coils (A. hiroshimensis, A. roseoverticillatus, A. rubroverticillatus, A. reticuli), or are spiral with many coils (from three to five or more).

In addition, our strain 1077 differs in that it never colors the medium. The spores are oval and spherical (in A. rubroverticillatus the spores are cylindrical). Strain 1077 differs from A. reticulus-ruber by its inability to inhibit the growth of the colon bacillus and in other properties (Table 4). Culture 1077 differs from A. aurantiacus in that the former produces verticillate sporophores.

SUMMARY

1. Actinomycete culture No. 1077, which differs from earlier described species of actinomycetes with verticillate structure of the sporophores, has been isolated from brown soil under cotton in the Peking region (CPR).

2. This culture forms verticillate sporophores; the whorls are primary and secondary. The sporophores are of medium length, and there are 0.5–1.0 coils on the end. The colonies are colored bright orange. The aerial mycelium is orange or pink. The pigment does not dissolve in water and belongs to the lipoactinochrome group.

3. The culture actively inhibits Gram-positive bacteria, mycobacteria, yeasts and yeast-like organisms as well as many species of fungi.

4. We have placed the culture described in a new species—Actinomyces aureoverticillatus n. sp.

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THE EFFECT OF THE SOURCE OF CARBON NUTRITION AND OF PHOSPHORUS ON STREPTOMYCIN BIOSYNTHESIS AND ON THE ACCUMULATION OF PYRUVIC ACID

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At the present time, the question of the role of phosphorus in the metabolism of the actinomycetes which produce antibiotics is attracting the attention of investigators.

According to data in the literature, an excess of phosphorus in the medium, while inhibiting the biosynthesis of chlortetracycline and oxytetracycline, also causes a number of substantial changes resulting in accelerated consumption of carbohydrates, the accumulation of pyruvic acid, and in the composition of nucleic acids in the mycelium (Biffi-Gentili, et al., 1954; Di Marco, et al., 1956; Doskočil, et al., 1958; Prokof'eva-Bel'govskaya, et al., 1959). The inhibition of streptomycin biosynthesis when media containing phosphorus in quantities exceeding its optimal concentration are used has also been noted by a number of authors (Hockenhull, et al., 1954; Brinberg and Grabovskaya, 1958). However, the effect of excess phosphorus on the process of pyruvic acid accumulation has not been studied in detail.

In the present work, the conditions of pyruvic acid accumulation as a function of the carbon source and phosphorus have been determined in connection with streptomycin biosynthesis.

EXPERIMENTAL PART

METHODS

Actinomyces streptomycini Kras., strain LS-1, was used in the experiments. The experiments were conducted under conditions of submerged growth in 750 ml Erlenmeyer flasks containing 100 ml of media; the flasks were placed on a shaker rotating at 220–230 rpm at 26–28°. The mycelium used for inoculum was grown on soy medium of the following composition (in %): soy meal–2.0; glucose–2.0; ammonium sulfate –0.3; sodium chloride–0.25; potassium phosphate–0.05; calcium carbonate–0.3. Inoculations were made with two-day mycelium grown on medium of the indicated composition in the amount of 2.0% by volume of the medium. The fermentation was carried out on two media: synthetic (Severin and Gorskaya, 1957) and corn medium of the following composition (in %): corn extract–0.5; glucose–2.0; sodium chloride–0.5; calcium carbonate–0.4; ammonium sulfate–0.4.

During the course of the experiment, samples were collected daily from three parallel flasks on which the following determinations were performed: weight of mycelium–by drying at 100–105° (traces of chalk were first dissolved in hydrochloric acid and the mycelium was washed with water); carbohydrates–by Shorl's method with preliminary hydrolysis in the case of starch; pyruvic acid by Friedemann's method, and in some experiments chromatographically; pH–electrometrically. Streptomycin was determined by the diffusion-in-agar method with Bacillus mycoides as the test microbe. Soluble inorganic phosphorus was determined by Kutner's method.

Average data of not less than three-five parallel experiments are given in the tables.

In connection with the fact that different batches of corn extract frequently differed in chemical composition and gave different results during biological testing, all experiments were conducted using a single batch of corn extract.

EXPERIMENTAL RESULTS

As early as 1957, it was found that, on media with corn extract, pyruvic acid accumulates in the first stages of development of A. streptomycini Kras., strain LS-1.

The indicated experiments were conducted in the presence of 2% glucose instead of the mixture of glucose and starch usually employed in corn medium. The use of glucose made it possible to raise the streptomycin content of the culture fluid to 2200 units/ml. In experiments with glucose mixed with starch, the activity of the culture fluid was only 1000 units/ml.

As is well known, when the organism is cultured on media with corn extract, its phosphorus requirement is satisfied by the phosphorus contained in corn extract.

In our experiments, this amount of soluble phosphorus constituted 7–8 mg %.

As seen from the data in Table 1, in the case where supplementary phosphorus (0.05%) was added, the accumulation of pyruvic acid nearly doubled in the first 24 hours of growth in comparison with the control. By the 48th hour of fermentation, the pyruvic acid was consumed and nearly the same amounts of it were found in the culture fluid in both cases. Along with this, a somewhat greater consumption of carbohydrates, a lowering of the reaction of the culture fluid, and an inhibition of streptomycin biosynthesis were observed in the experiments with added phosphorus.

Table 1. The Effect of Excess Phosphorus Content in the Medium on the Accumulation of Pyruvic Acid and on Streptomycin Biosynthesis (corn medium with glucose)

KH_2PO_4, %	Streptomycin, γ per ml			Pyruvic acid, γ per ml		Carbohydrates in medium, mg %		pH				Weight of mycelium, mg %			
Hours	48	72	96	24	48	24	48	24	48	72	96	24	48	72	96
Control (not added)	820	1606	2215	20.5	17.5	1649	762	6.9	6.6	7.1	7.3	150	399	502	430
0.05 (at inoculation)	510	1210	1603	39.3	19.0	1618	669	6.6	6.5	7.0	7.7	148	386	532	450

Table 2. The Effect of the Time of Phosphorus Addition on Streptomycin Biosynthesis, Pyruvic Acid Accumulation, and Consumption of Nutrient Substances (0.05% KH_1PO_4 was added)

Time of addition (hours)	Streptomycin (γ/ml, max)	Streptomycin biosyathesis inhib'n., %	Pyruvic acid, γ/ml, hr			Carbohydrates in medium, mg%, 48 hr	NH_3 – N in medium, mg%, 48 hr	pH, hours		Mycelium wt, mg %, hr	
			12	24	48			48	120	48	72
Control (not added)	2375	—	60.0	22.5	15.2	742	48.2	6.6	7.96	433	520
At inoculation	1575	34.5	70.0	35.0	17.1	634	32.5	6.5	8.0	498	589
After 12	1316	44.5	—	35.8	16.2	539	30.1	6.2	8.16	516	571
After 24	1580	33.7	—	—	—	720	42.3	6.7	8.2	435	542
After 48	2075	12.7	—	—	—	738	48.0	6.7	8.2	431	521

Table 3. Effect of the Carbon Source and Phosphorus Excess on Streptomycin Biosynthesis and Changes in the Medium (synthetic medium according to Severin and Gorskaya, 1957)

Carbon source	KH_2PO_4, %	Streptomycin γ/ml, hr			Medium carbohydrates, mg%, hr		Medium NH_3, mg%, hr		pH, hours			Mycelium wt, mg%, hr		
		72	96	120	24	48	24	48	24	48	72	24	48	72
Glucose	Control (not added)	900	1200	1600	1643	625	59.7	32.0	7.0	6.38	7.0	275	680	568
	0.05	580	1010	1200	1600	355	49.0	24.0	7.0	5.96	7.0	290	695	650
Starch	Control (not added)	280	380	350	1315	611	39.8	24.0	6.5	5.96	7.9	333	496	430
	0.05	225	380	400	1300	450	30.1	20.0	6.5	5.19	7.9	340	624	728

In connection with the data obtained, it was essential to determine the effect of the time of phosphorus addition on the accumulation of pyruvic acid and streptomycin biosynthesis. Data in the literature (Perlman and Wagman, 1952), according to which the addition of 0.1% phosphorus to soy fermentation medium at the time of inoculation inhibits streptomycin biosynthesis to the greatest extent, served as the basis for these experiments. According to the data of Di Marco et al. (1956), the inhibition of chlortetracycline biosynthesis is most strongly pronounced if phosphorus is added 12 hours after inoculation.

In order to clarify the given question, experiments were conducted in which potassium phosphate (0.05%) was added to the medium at the time of inoculation and after 12, 24, and 48 hours during the course of fermentation.

It was found (Table 2) that the supplementary addition of potassium phosphate to the medium in the amount of 0.05% at different intervals caused the inhibition of streptomycin biosynthesis in all cases, and that the greatest inhibition was observed when phosphate was added twelve hours following inoculation (nearly 50% inhibition as compared with the control), i.e., in the early stages of fermentation, while a less significant inhibition of biosynthesis was observed when phosphorus was added after 48 hours of fermentation.

The supplementary addition of phosphorus during the course of fermentation altered the character of development of the organism, namely: 1) increased mycelial weight when excess phosphorus was added at inoculation and after 12 hours of growth; 2) accelerated carbohydrate consumption in the same periods of time; 3) reduced the value of the hydrogen ion indicator; 4) inhibited streptomycin biosynthesis markedly.

As seen from Table 2, pyruvic acid accumulated in considerable quantities in the first hours of fermentation (12 hours), and then disappeared rapidly; by the 48th hour of growth, insignificant quantities of it remained in the culture fluid. When excess phosphorus was added at inoculation and after 12 hours of growth

somewhat larger quantities of pyruvic acid accumulated in the culture fluid as compared with the control.

As is well known, the production of streptomycin using strain LS-1 proceeds most intensively when glucose is employed as the carbon source. When the latter is replaced by starch, considerable reduction in streptomycin biosynthesis is observed (Surikova, 1959).

In connection with the fact noted, it was essential to determine the relationship between streptomycin biosynthesis and pyruvic acid accumulation in the presence of starch. The effect of excess phosphorus (0.05% potassium phosphate) on the given process was determined at the same time. The given series of experiments was carried out on a synthetic medium. In experiments with starch, 0.5% glucose and 1.5% starch was used instead of 2% glucose.

Medium with glucose was tested as the control.

From the data presented in Table 3, it is seen that in the experiments with starch, streptomycin production occurred at a considerably lower level as compared with glucose. The inhibition of streptomycin biosynthesis was accompanied by a number of metabolic changes. First of all, it should be noted that, despite the high rate of biomass accumulation in the first 24 hours of growth in the experiments with starch, the maximal weight of the mycelium after 48 hours of growth was lower than under the same conditions in the presence of glucose. More vigorous consumption of ammonium nitrogen and carbohydrates was observed in the experiments with starch.

When glucose was used, ensuring rather high streptomycin yields, pyruvic acid accumulation took place at the first stages of development (12 hours of growth), its amount gradually decreasing later on, while in the presence of starch, pyruvic acid accumulated in considerable concentrations, reaching a maximum by the 24th hour of fermentation.

When the amounts of accumulated pyruvic acid are compared, it is seen that many times more of it was found in the last case than in experiments with glucose (Fig. 1).

The addition of excess phosphorus to the medium somewhat increased the accumulation of pyruvic acid in the presence of glucose; no such effect was found with starch (Fig. 2).

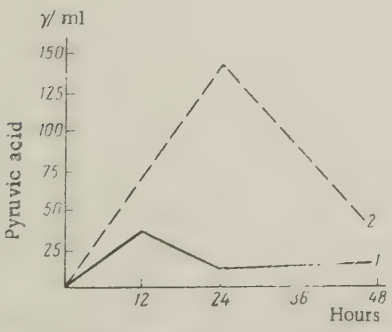

Fig. 1. Accumulation of pyruvic acid during the course of fermentation as a function of the carbon source. 1) On glucose; 2) on starch.

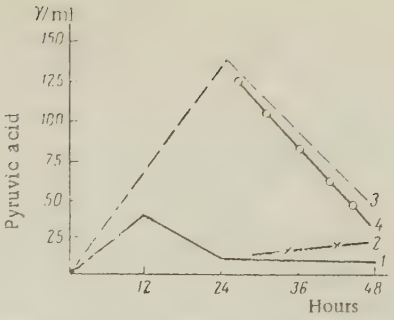

Fig. 2. Effect of supplementary addition of phosphorus during the course of fermentation on the accumulation of pyruvic acid. 1) On glucose; 2) on glucose + 0.05% KH_2PO_4 after 24 hours; 3) on starch; 4) on starch + 0.05% KH_2PO_4 after 24 hours.

The patterns found provide a basis for offering certain considerations of the following character.

The accumulation of considerable amounts of pyruvic acid took place in the presence of starch; on glucose, practically no pyruvic acid accumulation occurred.

If these data are considered in connection with the simultaneously occurring process of streptomycin biosynthesis, the impression is created that the sharp reduction in streptomycin production in the experiments with starch is apparently explained to some extent by considerable pyruvic acid accumulation, i.e., under these conditions, pyruvic acid is consumed more slowly. The course of the pyruvic acid curve in the experiments with glucose is evidence of its utilization by the organism, which apparently provides metabolic conditions favorable for the normal progress of streptomycin synthesis. According to data in the literature, these patterns also occur for other actinomycetes, particularly for the producer of erythromycin (Musílek and Ševčík, 1958).

Thus, the investigations conducted made it possible to detect certain patterns in the streptomycin producing actinomycete which evidently point to a connection between the process of pyruvic acid oxidation and streptomycin biosynthesis.

SUMMARY

The formation of pyruvic acid was studied in the course of fermentation with respect to the kind of carbon source and phosphorus excess in the medium.

It was shown that under conditions ensuring a high yield of streptomycin, in fermentations with glucose, pyruvic acid is accumulated in insignificant amounts only at the beginning of the process, being rapidly consumed thereafter.

In the presence of starch, appreciable accumulation of pyruvic acid accompanied by a marked decrease of streptomycin biosynthesis is observed.

An addition of excess phosphorus (0.05% KH_2PO_4) inhibits the biosynthesis of streptomycin, particularly during early growth and development of the organism.

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THE RELATION OF INDIVIDUAL FORMS OF SOIL ACTINOMYCETES TO VARIOUS CARBON SOURCES DURING GROWTH ON NITRATE AND MOLECULAR NITROGEN

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In our preceding communications (Fedorov and Kudryashova, 1956; Fedorov and Il'ina, 1959), experimental data were presented on the ability of certain soil actinomycetes to fix molecular nitrogen from the atmosphere. Similar data have been published by other investigators as well (Plotho, 1940; Panosyan, 1945). However, the productivity of the given process in these organisms was very low. Even the most active fixers of atmospheric nitrogen in the actinomycete group assimilate only 3–4 mg of molecular nitrogen per 1 g of glucose utilized, while the less active forms assimilate only 1–2 mg. The addition of yeast autolyzate to the nitrogen-free nutrient medium usually increases the productivity of fixation in these microorganisms (Fedorov and Il'ina, 1959). It must therefore be assumed that actinomycetes growing at the expense of molecular nitrogen require some additional growth factors.

In connection with the fact that their nitrogen-fixing activity is very poor, it would be desirable to know whether the source of the energy-yielding substance consumed by them has any effect on this process. When the chemical structure of the oxidizable energy-yielding substance varies, the rate of mobilization of hydrogen from it undergoes large fluctuations and may have an effect on the productivity of the fixation of atmospheric nitrogen.

It was for the purpose of clarifying this question that we undertook experiments in which actinomycetes capable of fixing atmospheric nitrogen were cultured on various carbon sources both in the presence of bound nitrogen compounds in the medium (nitrates) and without them.

Part of our experiments were set up on nitrogen-free medium as modified by Fedorov to which the appropriate carbon source was added in a concentration of approximately 0.05 moles.* The test included 24 organic compounds with various chemical structures. Cultivation was carried out in a special incubator at 30° for 30 days. At the end of the experiment, the amount of unused energy-yielding substance was determined in the medium (by specialized methods), as well as the amount of nitrogen accumulated due to the fixation of atmospheric nitrogen (by the Kjeldahl method).

The results of these analyses are given in Table 1.

The data in Table 1 show that various actinomycetes behaved differently with respect to the different carbon sources. Some of them grew on the majority of the sources studied, while others grew only on some of them. Such carbon sources as lactose, maltose, glycerol, sorbitol, formic, fumaric, tartaric, citric, quinic, and benzoic acids proved to be unsuitable or only slightly suitable for all of the actinomycete cultures studied which were growing at the expense of molecular nitrogen. However, even in those cases where the carbon source was consumed by them, the increment of nitrogen in the medium was not great in the majority of cases. The most favorable carbon sources for the assimilation of atmospheric nitrogen were sucrose, mannitol, succinic acid, and starch. On starch, the increment of nitrogen reached 0.3 mg per 30 ml of medium, while the consumption of starch was as high as 40% of the amount contained in the medium.

In connection with the fact that the actinomycetes failed to grow on a number of carbon sources, the reason for the absence of growth had to be determined. It may have been associated either with the inaccessibility of the given organic compound as a source of carbon, or with the peculiarities of their growth when using molecular nitrogen. To resolve this question, a second experiment was set up with the same carbon sources, but with the addition of sodium nitrate to the medium as a source of bound nitrogen. The results of this experiment are given in Table 2.

The data in Table 2 show that on medium with nitrate as well, many carbon sources (formic, fumaric, tartaric, citric, and benzoic acids) remain unavailable for the actinomycetes which we studied. Although sorbitol and quinic acid were partially utilized by some of them, they were either entirely unsuitable or only slightly suitable carbon sources for most of them. These microorganisms grew well on other organic compounds with rather varied chemical structures if the medium contained nitrate (lactic, pyruvic, and succinic acids, mannitol, glycerol, xylose, arabinose, dextrin, maltose, lactose, sucrose, glucose, fructose, and galactose), but they did not all serve as good energy-yielding material for the fixation of molecular nitrogen. Thus, for example, on xylose, fructose, and succinic acid, the productivity of nitrogen fixation was very slight and the consumption of these carbon sources was insignificant. If bound nitrogen was added to the medium, however, their consumption rose sharply and they became good carbon sources for the actinomycetes being tested. This

*As in original—Publisher's note.

Table 1. The Nitrogen-Fixing Activity of Individual Strains of Actinomycetes on Nitrogen-Free Medium Containing Various Carbon Sources

Carbon source	Actinomycete strains	Amount of carbon source consumed, in mg		Amount of carbon source consumed, in %		Increment of N per 30 ml medium, mg		Productivity of N fixation per g C source consumed, mg	
		1	2	1	2	1	2	1	2
Glucose, 210 mg	39	14	40	6.66	19.04	—	0.12	0.0	3.0
	78	10	10	4.76	4.76	0.05	—	5.0	0.0
Fructose, 240.2 mg	39	11.9	—	4.95	—	0.01	0.28	0.84	—
Galactose, 245.1 mg	39	0.9	—	0.36	—	0.25	0.08	—	—
Sucrose, 257.7 mg	39	—	33.7	—	13.07	0.14	0.17	—	5.04
	78	15.7	6.7	6.09	2.60	0.18	0.16	11.46	23.88
Starch, 172 mg	49	79	59	45.93	34.30	0.32	0.37	4.51	6.27
	78	42	34	19.76	22.09	0.30	0.29	7.14	8.52
Dextrin, 149.4 mg	39	9.4	—	6.29	—	0.04	0.13	4.25	—
Arabinose, 201.7 mg	39	11.0	—	5.45	—	0.13	0.01	11.82	—
Xylose, 221.3 mg	39	10.9	—	4.92	—	0.02	--	1.83	—
Mannitol, 264 mg	39	35	49	13.25	18.56	0.12	0.13	3.42	2.65
	78	40	31	15.15	11.74	0.15	0.08	3.75	0
Acetic acid, 262.95 mg	39	14.5	19.1	5.51	7.26	0.14	0.10	9.65	5.23
Pyruvic acid, 58 mg	39	6.0	—	10.34	—	0.0	0.09	0.0	—
Succinic acid, 442.32 mg	39	87.2	167.6	19.71	37.89	—	0.13	—	0.77

peculiarity of their behavior is of considerable scientific interest and requires further, more detailed investigation. Since oxidative processes in the microbial cell begin with the mobilization of hydrogen by the appropriate dehydrogenases (the hydrogen being further oxidized to water by molecular oxygen and partially utilized for the reduction of molecular nitrogen to amino compounds), the absence of growth of the test actinomycetes on the indicated organic compounds at the expense of the utilization of molecular nitrogen suggests that the presence of bound nitrogen in the medium is evidently required for the synthesis of these dehydrogenases.

As shown by the data given, even such a widely distributed enzyme as succinic dehydrogenase, which is very active in the cells of actinomycetes growing in the presence of nitrate, and which oxidized more than 400 mg of succinic acid during the course of the experiment, was only slightly active in the cells of actinomycetes growing without bound nitrogen in the medium and oxidized only 87–167 mg of this acid, i.e., three times less. Similar results were obtained with xylose as well. In the presence of nitrate in the medium, 221 mg of xylose was oxidized, but only 109 mg without the presence of bound nitrogen in the medium. Nearly the same result was obtained with growth on fructose: 240 mg of fructose was oxidized in medium containing nitrate, but only 11.9 mg in medium without bound nitrogen. This result is difficult to associate with any peculiarity of chemical structure of these compounds. In addition to this, it must be noted that although the actinomycetes tested grew several times faster on bound nitrogen, they synthesized their cellular substance less productively. This situation was confirmed by the data on the nitrogen content of the mycelium of actinomycetes grown on various nitrogen sources. When growing on nitrate nitrogen, they accumulated 0.9–1.8 mg of cellular nitrogen in the mycelium per 240 mg of fructose oxidized, but when growing on molecular nitrogen, they accumulated 0.28 mg of cellular nitrogen per 11.9 mg of fructose oxidized. In the first case, the ratio between carbon oxidized and nitrogen assimilated was about 75 : 1, while in the second case—about 20 : 1. During growth on nitrate nitrogen, part of the hydrogen mobilized was evidently expended for the useless reduction of nitrate, since 0.9–1.8 mg of nitrogen was utilized for the synthesis of cellular material, while 5.87 mg of nitrate nitrogen disappeared from the medium. Metabolism evidently proceeds along different pathways with different nitrogen sources, and this affects the productivity of utilization of the energy-yielding substance. The conversion of a microorganism with a poorly developed capacity for fixing atmospheric nitrogen to nutrition involving molecular nitrogen obviously causes drastic changes in metabolism, particularly in the course of oxidative processes and in the activity of enzymes participating in these processes. In real molecular nitrogen fixers, however, such sharp differences are usually not observed. Therefore, a detailed study of the physiological properties of weak nitrogen fixers . . .†

SUMMARY

1. When certain actinomycetes in which the capacity to fix molecular nitrogen is poor are cultured on nitrogen-free medium and on the same medium with added nitrates, it was established that they behav

†A typographical error appears in the Russian journal. The last li or lines of the paragraph is missing, and for it, part of a previous li (two lines above it) has been substituted—thus not making a reasonab sentence.—Translator's note.

Table 2. The Growth of Actinomycetes on Various Carbon Sources in the Presence of Bound Nitrogen in the Medium (sodium nitrate)

Carbon source	Actinomycete strains	Final pH	C source consumed		Mycelium wt, mg	Mycelium % C source consumed	Mycelium N content		$N(NO_3)$ consumed	
			mg	%			mg	% dry wt	mg	%
Glucose, 210 mg	49	7.8 7.5	210 210	100.0	22.2 23.7	10.92	0.53 0.97	3.23	5.68 5.68	100.0
Fructose, 240.2 mg	49	8.8—8.7 8.8	240.2 240.2	100.0	44.9 29.4	15.46	0.90 0.89	2.51	5.74 5.74	100.0
	78	8.6—8.7 8.6—8.7	240.2 240.2	100.0	37.2 41.1	16.29	1.80 1.71	4.49	5.74 5.74	100.0
Galactose, 245.1 mg	49	8.6 8.6	245.1 245.1	100.0	15.9 24.5	8.23	0.67 0.89	3.92	5.74 5.74	100.0
	78	8.0 8.0	245.1 245.1	100.0	33.7 37.4	14.49	1.86 1.44	4.68	5.74 5.74	100.0
Sucrose, 257.7 mg	10	7.0 7.0	257.7 257.7	100.0	14.2 10.3	4.75	0.58 0.32	3.59	5.68 5.58	100.0
	49	7.0	181.55 257.70	85.22	15.9 37.2	11.59	1.08 1.33	5.18	5.68 5.68	100.0
Lactose, 236.1 mg	39	8.5—8.8 8.7—8.8	236.1 236.1	100.0	40.2 39.1	16.79	1.17 1.32	3.14	5.74 5.74	100.0
	49	8.5—8.8 8.5—8.8	236.1 236.1	100.0	24.2 22.3	9.84	1.01 0.94	4.19	5.74 5.74	100.0
	78	7.8 8.5	236.1 236.1	100.0	35.0 39.0	15.66	1.97 1.96	5.32	5.74 5.74	100.0
Maltose, 250.8 mg	10	7.7—7.8 —	250.8 250.8	100.0	17.1 —	6.81	0.67 —	3.91	5.74 5.74	100.0
	39	8.7—8.8 8.7—8.8	250.8 250.8	100	27.0 30.1	11.38	1.67 0.99	4.58	5.74 5.74	100.0
	49	8.6—8.8 8.8	250.8 250.8	100.0	22.0 21.1	8.59	1.20 1.13	5.40	5.74 5.74	100.0
	78	7.5 8.8	250.8 250.8	100.0	36.0 30.4	13.03	1.81 1.82	5.57	5.74 5.74	100.0
Dextrin, 149.4 mg	10	8.8 —	149.4 —	100.0	11.9 —	7.96	0.13 —	1.09	5.74 —	100.0
	49	8.8 8.8	149.4 149.4	100.0	31.0 31.5	20.91	0.99 0.84	2.92	5.74 5.74	100.0
	78	8.5—8.8 8.5—8.8	149.4 149.4	100.0	31.6 32.4	21.41	2.22 2.33	7.13	5.74 5.74	100.0
Arabinose, 201.7 mg	10	6.5 —	201.7 —	100.0	14.6 —	7.23	0.59 —	4.04	4.31 —	100.0
	78	6.0—6.1 6.0—6.1	201.7 201.7	100.0	4.7 5.0	2.40	0.31 0.32	6.49	4.31 4.31	100.0
Xylose, 221.3 mg	10	6.4 —	221.3 —	100.0	9.8 —	4.47	0.85 —	8.58	4.31 —	100.0
	49	7.6—7.8 7.6—7.8	221.3 221.3	100.0	23.2 14.9	8.60	0.54 0.46	2.70	4.31 4.31	100.0
	78	7.6—7.8 7.6—7.8	221.3 221.3	100.0	30.1 33.3	14.32	1.61 1.70	5.22	4.61 4.70	100.0
Glycerol, 249 mg	39	8.8	249	100.0	28.6 30.5	11.86	0.93 0.99	3.24	5.74	100.0
	49	8.8	249	100.0	27.7 28.1	11.20	1.22 1.32	4.54	5.74	100.0
	78	8.0	249	100.0	34.1 32.8	13.43	1.57 1.54	4.64	5.74	100.0

Table 2 (continued).

Carbon source	Actinomycete strains	Final pH	C source consumed (mg)	C source consumed (%)	Mycelium wt, mg	Mycelium %C source consumed	Mycelium N content mg	Mycelium N content % dry wt	N (NO_3) consumed mg	N (NO_3) consumed %
Mannitol, 264 mg	49	7.8	243.3	92.04	33.8	13.91	1.41	4.17	5.68	100.0
			—		—		—		—	
Sorbitol, 210 mg	39	7.3—7.4	—	—	10.0	—	0.13	1.05	3.11	56.70
					13.5		0.11		3.40	
	78	7.3—7.4	—	—	18.5	—	0.08	0.64	2.76	50.52
		7.2—7.3			26.5		0.23		3.05	
Pyruvic acid, 58 mg	49	8.8—9.0	18.0	31.58	5.3	30.55	0.12	2.88	2.01	37.19
					5.7		0.20		2.26	
	78	8.8—9.0	12.0	21.05	7.2	65.83	0.35	4.93	2.09	36.41
					8.6		0.43		2.09	
Lactic acid, 193.9 mg	49	7.3—7.4	134.67	72.88	76.9	56.86	0.51	0.83	3.57	63.50
			147.97		83.8		0.85		3.72	
	78	7.0	—	—	57.5	33.1	0.76	1.35	3.23	56.79
					71.0		0.99		3.29	
Succinic acid, 442.32 mg	10	9.8	415.07	94.17	19.7	4.63	0.87	3.81	5.11	88.37
			418.05		18.9		0.61		4.93	
	78	9.3	383.66	88.45	20.6	4.65	0.37	2.50	3.74	65.13
			399.85		15.8		0.51		3.66	
Quinic acid, 224.01 mg	39	7.5—7.6	91.44	44.74	4.4	3.87	0.20	6.33	3.38	55.05
		7.5—7.6	109.04		3.2		0.26		2.94	

differently toward different carbon sources and show different productivity in utilizing them for the synthesis of cellular material. On succinic acid, xylose, fructose, and certain other compounds, the oxidation rate proved to be 2–3 times higher during growth on nitrate nitrogen, while the productivity of synthesis of cellular material was 2–3 times lower.

2. The conversion of a microorganism which has a poorly developed capacity for fixing molecular nitrogen to nutrition involving molecular nitrogen causes sharp changes in metabolism, particularly in the activity of oxidative processes and in the processes of synthesis of cellular material. This phenomenon is usually not observed in microorganisms which fix molecular nitrogen actively.

3. The presence of bound nitrogen compounds in the medium is important for the synthesis of some dehydrogenases. Without them, they either exhibit no activity at all, or it is very slight.

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THE MORPHOLOGY OF AMINO ACID DEFICIENT VARIANTS OF ASPERGILLUS NIDULANS AS A FUNCTION OF THE COMPOSITION OF THE MEDIUM

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In the preceding work (Kasatkina, 1959), it was shown that certain morphologically altered Aspergillus nidulans variants which were obtained through the effect of ultraviolet irradiation are at the same time biochemical mutants which have lost the ability to synthesize various metabolites. Mutants of this type are especially fastidious with regard to nutrient medium; their growth and normal development depend on the presence in the medium of the substances whose synthesis was impaired. A detailed description of the morphology of A. nidulans variants produced with different doses of ultraviolet irradiation and their classification on the basis of morphological differences is given in the works of Brotskaya (1958) and Kuzyurina (1959).

This communication, dwells only upon certain characteristic morphological properties of A. nidulans variants which had lost their ability to synthesize certain amino acids through the effect of irradiation, comparing them with the original form of the fungus which had not been subjected to irradiation.

METHODS

Seven amino acid deficient variants of A. niduians were used in the work. Two of them required arginine for growth, three variants were deficient in tryptophane, one required lysine, and one required methionine. The method of obtaining the given variants and their properties have been given earlier (Kasatkina, 1959).

Inasmuch as deficient mutants exhibit special requirements with respect to the nutrient medium, the latter has to be richer not only in its qualitative composition, but also in its quantitative content of those substances which are essential for the growth of the mutant. Moreover, the assumption was made that the altered morphology of deficient A. nidulans variants observed on wort agar is possibly the result of their growth on a medium which does not fully satisfy their new nutritional requirements.

Proceeding from these considerations, the following nutrient media were employed for studying the morphology of amino acid deficient variants of A. nidulans: wort agar (7° Balling) as the basic medium, and wort agar enriched with the individual amino acids which the particular deficient variants required. Each of the amino acids was added in the form of a sterile solution in wort agar to give 1 mg per ml of medium. Giant colonies of the fungus cultures were grown on the surface of these media in Petri dishes, and observations of the character of their growth and development were made. The fungi were grown for ten days at 24°. The growth rate of the colonies, the topography of the surface and structure of the colony, the color, pigment production, etc., were noted.

The character of mycelial growth and conidia formation of the variants was observed in the dynamics of growth of the colonies. For microscopic study, small pieces of mycelium were removed from various parts of variant colonies of different ages.

RESULTS

In distinction from the rapidly growing, large, dark green (due to profuse conidia production) colonies of the wild form of A. nidulans, the colonies of amino acid deficient variants grow more slowly on wort agar, are smaller in size, and are white or brown in color. Variants deficient in arginine or in lysine resemble one another very closely in colony morphology, and differ from the wild form and from other deficient variants by the production of brown pigment which colors the mycelium and the agar around their colonies brown. In addition to this, due to the vigorous development of the aerial mycelium, their colonies have a fluffy surface in distinction from the smooth colony surface of the original form and other variants.

When grown on wort agar, tryptophane-requiring variants are distinguished by particularly poor growth. They characteristically form flat, white, almost transparent colonies of very loose structure. These colonies have a considerably smaller diameter than colonies of the original form. The methionine-deficient variant forms dense, white colonies with radial folds. Figure 1 gives an idea of the colony morphology of the wild form of A. nidulans and of the arginine- or tryptophane-deficient variants grown on wort agar (ten-day-old colonies shown in natural size).

The results of the microscopic study of mycelium taken from variant colonies of different ages points to one common trait characteristic for all of the forms studied. This peculiarity consists of the fact that the development of the variants on wort agar does not quite follow the usual course; it does not terminate in profuse conidia production as is the case in the

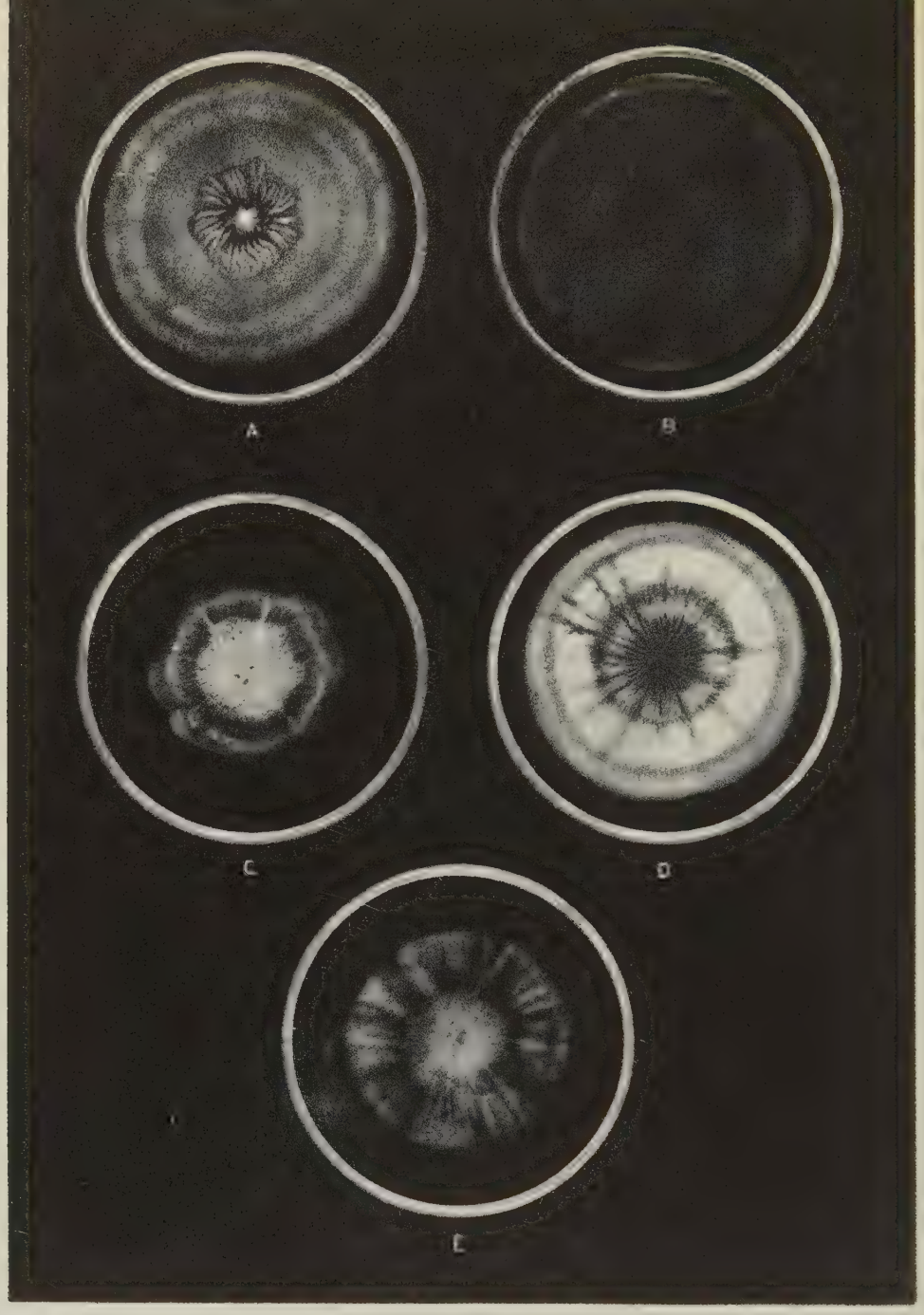

Fig. 1. Giant colonies of Aspergillus nidulans. A) Original culture on wort agar; B) arginine-deficient variant on wort agar; C) tryptophane-deficient variant on wort agar; D) arginine-deficient variant on wort agar + deficient amino acid; E) tryptophane-deficient variant on wort agar + deficient amino acid.

Figure 1 was printed in color in the original Russian. Technical difficulties both necessitate its being rendered into black and white and cause the loss of clarity between different areas. A color description follows.

A) Center off-white to ochre. Radiating lines pale green on deep green background. First inside ring dark green, becoming lighter in two outside rings. Occasional specks of brown.

B) Mottled reddish-brown, ochre, and dark green coloring.

C) Off-white center with brown specks. Brown second ring. Off-white second and third rings. Radiating lines from center off-white.

D) Dark green center with dark green radiating lines becoming paler at the outside rings. Second ring light green. Third ring dark green. Fourth ring pale green. Fifth ring from dark to light green.

E) Center pale-blue—pale-green with brown specks, turning dark green and dark blue in second ring. Outside ring alternate bands of brown, ochre, light blue, and light and dark green.

original form. When the mycelium of the variants is examined microscopically, it can be seen that the conidiophores branching off from the mycelial hyphae are underdeveloped in most cases; their development is frequently limited to the formation of an apical vesicle which is either devoid of sterigmata or bears one row of sterigmata with single conidia. On this basis, the A. nidulans variants deficient in the amino acids mentioned and grown on wort agar can be related to the aconidial or oligoconidial type.

Entirely different morphology of the deficient variants was observed when they were grown on wort agar to which the amino acids essential for the growth of the variant were added. Giant colonies of the corresponding deficient variants were grown on the surface of wort media containing arginine, lysine, tryptophane, or methionine.

In their outward appearance, the colonies of variants requiring arginine, lysine, or tryptophane grown on wort agar with amino acids hardly differed from colonies of the original form obtained on wort agar (control). They increased in size, acquired a denser structure, and were of a green color, just the same as the colonies of the original form (Fig. 1). On this medium, arginine- and lysine-deficient variants no longer produced brown pigment. Microscopic data on the mycelium of the variants showed that their development on these media proceeded normally and terminated with intensive conidia formation just as in the original form. The methionine-deficient mutant behaved somewhat differently. Different doses of methionine added to the wort agar did not eliminate its morphological distinctions from the original form. The previously observed (Kasatkina, 1959) poor growth of this mutant on minimal medium with methionine is evidence of its requirement for some additional substances besides methionine. Its weak response to the addition to wort of methionine alone is probably connected with this circumstance. In the rest of the cases, the "abnormal" morphology of A. nidulans variants was evidently due to the inadequate content in the medium of the particular amino acid for which the mutant had lost its synthetic ability through the influence of irradiation.

In order to arrive at some idea of the amounts of amino acid which exert a stimulatory effect on the growth of deficient variants on a background of wort

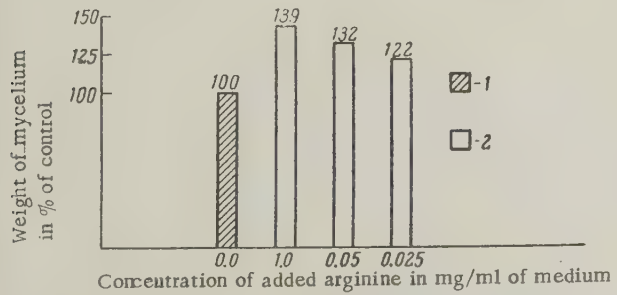

Fig. 2. Mycelial weight of arginine-deficient variant of Aspergillus nidulans on wort with added arginine. 1) Mycelial weight without added arginine (control); 2) mycelial weight at different concentrations of arginine.

medium, experiments were conducted with an arginine-requiring variant. For this purpose, an arginine-deficient variant was grown on the surface of liquid wort (7° Balling) in 50 ml Erlenmeyer flasks at 24°. The volume of the medium was 20 ml. The experiments were carried out with various batches of wort. An arginine solution, calculated to give a concentration of 1 mg/ml, 50 μ g/ml, and 25 μ g/ml of medium, was placed under young two-day-old pellicles of the fungus. No amino acid was added to the control flasks. The dry weight of the mycelium which had grown up was determined on the sixth day of growth. The composite data of five experiments are presented in Fig. 2. It can be seen that the addition to wort of even minute amounts of arginine, expressed in micrograms (25 μ g) led to an approximately 20% increase in the mycelial weight of the deficient variant as compared with the control pellicle which had grown on wort without added amino acid.

Despite the absence of a single theory at the present time which would explain the various facts of morphological variability of microorganisms, it can be assumed that this variability is the result of changes occurring in the nutritional physiology of the organism. This position is illustrated by the mutants of A. nidulans, in which the disruption of normal physiology consists of the loss of their ability to synthesize amino acids. If the physiological deficiency of the given variants is eliminated by providing them with the necessary amounts of the deficient amino acid, in other words, if the variants are grown under such conditions that their metabolism goes on normally, then neither can any change in their morphology be observed.

The data obtained once again emphasize the importance of the fact that one cannot make judgements concerning hereditary morphological changes arising in a microorganism under the influence of irradiation if it is only grown on any one nutrient medium. This medium may not satisfy the new nutritional requirements of the variant and may in this way distort the true picture of its morphology. In this connection, it should be noted that wort agar (7° Balling), which is usually employed in the selection of microorganisms, is evidently not an entirely complete medium for fungal mutants which require amino acids. The growth of variants deficient in various amino acids observed on this medium speaks for its suitability with respect to the qualitative amino acid content. However, the limited growth of the variants and their abnormal development on this medium, which is eliminated by the addition of the deficient amino acid, is evidence of its deficiency in the quantitative content of certain amino acids. On the other hand, wort agar, on which amino acid deficient variants of A. nidulans differ so markedly in their morphology from the original non-deficient culture, serves as a good diagnostic medium for their more rapid selection according to morphological characters.

I should like to offer my gratitude to Prof. A. A. Imshenetskii for critical comments during the performance of the given work.

SUMMARY

1. According to the morphology of their colonies on wort agar, the arginine, lysine, tryptophane, or methionine deficient variants of Aspergillus nidulans can be related to aconidial or oligoconidial variants.

2. The morphological changes are levelled off in mutants cultured on wort agar to which the deficient amino acid is added. The latter improves growth of the mutant and stimulates the formation of conidia.

3. The results obtained confirm the general notion that morphological variability is based on changes in the physiology of the organism. The conditions of the medium which eliminate the physiological incompleteness of the variant ensure the restoration of its normal morphology as well.

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TRANSFORMATION CARRIED OUT BY CELL-FREE EXTRACTS

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The phenomenon of transformation or the transmission of certain properties in bacteria with the aid of desoxyribonucleic acid (DNA) is being studied from a great variety of approaches. The effect on transformation of the proteins contained in nutrient medium is being clarified; the specificity of DNA is being demonstrated by the fact that, following the action of the enzyme desoxyribonuclease, the transforming ability of DNA weakens or disappears; the degree of polymerization of DNA is being contrasted with its transforming activity; etc.

For the general microbiologist, the question of the possibility of bringing about transformation with the aid of the products formed during the disintegration of bacterial cells is of considerable interest. For many decades, the symbiosis of bacteria has figured as one of the factors in variability. Ordinarily the changes taking place have been explained by the action of the products of vital activity on the culture, by a change in the reaction of the medium, the effect of pigments or antibiotics, etc. Now, when there is every reason to believe that cultures of symbionts always contain the DNA of both strains, released during the dying off and disintegration of cells, it can naturally be supposed that in a number of cases the observed variability is associated with the action of "heterogenic" DNA. The question of whether the observed effect is connected only with the action of DNA or whether other cellular components take part in this as well remains open. For the biologist, these questions are of exceptional interest, since only in the bacterial world is it possible to have spontaneous partial exchange of cell content leading to hereditary changes.

In our preceding communication (Imshenetskii et al., 1959), the results of investigations on the transformation of a staphylococcus were reported. By passing the staphylococcus on media containing constantly increasing concentrations of streptomycin, a strain resistant to streptomycin was obtained. The DNA was extracted from the cells of this strain, and various amounts of it were added to medium which was inoculated with the original sensitive staphylococcus strain. The staphylococcus from these cultures was then plated on solid, streptomycin-containing medium. It was found that resistant variants of the staphylococcus were produced in considerable numbers as the result of the action of DNA.

The problem was then posed of bringing about the production of such resistant forms by treatment not with DNA, but with extracts obtained from the cells of streptomycin-resistant staphylococcus. It is the description of thes investigations that is presented in the given communication.

EXPERIMENTAL PART

METHODS

The experiments were conducted with the original culture, Staphylococcus aureus 209, and with an experimentally obtained strain of the same staphylococcus which is resistant to streptomycin in a concentration of 40,000 units/ml of nutrient medium. This resistant strain was cultured in flasks or carboys with a capacity of 10 liters. The staphylococcus was grown on meat-peptone broth containing 10,000 units/ml streptomycin. The carboys and flasks were kept in an incubator at 37° for 24 hours. The staphylococcus cells were then separated out, washed with distilled water, and 30 ml of 37 1/2% ethyl alcohol was added to them. The staphylococcus cells were stored in a refrigerator at 5° for 18 hours. Later, the ethyl alcohol was completely removed from the cells and they were ground with Schott glass in an agate mortar for one hour in the cold. Fifteen ml of physiological saline solution was added to the disrupted cells. This mixture was then centrifuged for 35 minutes at 17,000 revolutions, and the supernatant obtained was filtered through a dense double paper filter. The filtrate was poured into small test tubes and centrifuged again for 20 minutes at 12,000 rpm. The surface layer was carefully suctioned off and the experiments were done with this fluid. The supernatant fluid was inoculated into liquid and solid meat-peptone media in order to demonstrate that it contained no viable cells of the resistant staphylococcus. Aside from this, the presence of DNA in the supernatant fluid, which will henceforth be designated as cell-free extract, was established qualitatively by means of the Feulgen reaction. In this manner it was demonstrated that each cell-free extract with which experiments were carried out had no live staphylococcus cells and contained DNA.

EXPERIMENTAL RESULTS

The experiments began with the determination of the number of streptomycin-resistant cells present in the original culture which had not been adapted to streptomycin. At first, an inoculation was made on slanted meat-peptone agar in test tubes which contained 40,000 units/ml of streptomycin. Such a streak inoculation gave no growth. However, these results were not convincing, since the frequency with which streptomycin-

Table 1. Number of Streptomycin-Resistant Cells in the Original Staphylococcus Culture (duration of experiments 36 hours)

Experiment No.	Number of cells which grew up on MPA with streptomycin		
	Number of cells inoculated on plates	Number of streptomycin-resistant cells	Number of cells giving one mutation
1	320 000 000	1	$0.32 \cdot 10^9$
2	15 000 000 000	600	$0.25 \cdot 10^9$
3	9 680 000 000	15	$0.65 \cdot 10^9$
4	14 720 000 000	25	$0.60 \cdot 10^9$
5	2 896 000 000	12	$0.25 \cdot 10^9$

resistant variants arose could have been so insignificant that cells of resistant variants could have been absent from the inoculum placed on the surface of the agar in the test tube. Therefore, from then on, considerable numbers of cells of the nonresistant staphylococcus were simultaneously plated on a very large number of Petri dishes of MPA containing 40,000 units/ml of streptomycin. The results of the experiments are given in Table 1.

These data make it possible to claim that the cell population in the original staphylococcus culture contained a very small number of streptomycin-resistant cells. With the exception of the second experiment, in which there were considerably more such cells, the number of cells giving one mutation in the remaining four experiments fluctuated from $0.25 \cdot 10^9$ to $0.65 \cdot 10^9$.

To determine the ability of the cell-free extract to bring about transformation, it was added to meat-peptone broth in which the original streptomycin-sensitive strain of staphylococcus was grown. The inoculum was added from solid nutrient medium in amounts such that 1 ml of nutrient medium would contain from 10,000,000 to 20,000,000 staphylococcal cells. The inoculated flasks were kept in an incubator at 26° for 24–36 hours. After this period of time, the flask contents were plated on Petri dishes of MPA containing 40,000 units of streptomycin per 1 ml. The inoculated plates were kept in an incubator at 37° for 48–72 hours. The number of colonies growing up on the plates was counted; their number corresponded to the number of resistant variants.

Thus, only colonies of resistant variants grew on the plates with streptomycin. In order to determine the number of cells per plate added in these inoculations, parallel inoculations were made with the same amount of culture on Petri plates of MPA without streptomycin. By counting the colonies growing up on this medium and by comparing their number with that of the colonies appearing on plates of streptomycin agar, it was possible to arrive at some idea of the number of mutations. In these experiments, the amount of extract added to MPB was varied, and the final dilution of extract in the medium was 1:2, 1:5, 1:10, 1:100. The numerical material given in Table 2 enables us to conclude that the maximal number of resistant forms is produced at an extract dilution of 1:10. In this case, the number of cells giving one mutation reached $0.14 \cdot 10^6 - 0.2 \cdot 10^5$. It should be recalled that in the original population, resistant forms were encountered in amounts of $0.25 - 0.65 \cdot 10^9$. Consequently the number of resistant forms increased 1000 or 10,000-fold through the effect of the extracts.

In connection with this, the question arose: How soon does the formation of resistant variants occur in the original culture under the influence of extract obtained from the cells of the resistant variant? In order to answer this question, inoculations were made from the staphylococcus culture growing in the presence of extract on plates of streptomycin agar after 5, 18, 26, and 36 hours. The results of these experiments are given in Table 3. They can be summarized as follows: 1) Growth of the culture for five hours was not accompanied by the production of resistant mutations. 2) There were already many resistant variants in 18-hour-old cultures, and further increase in their number in older cultures was relatively insignificant. 3) The number of mutations increased considerably—one mutation occurred per $0.45 \cdot 10^2$ cells. 4) No transformation was observed when extract was used in a 1:100 dilution.

Theoretically, it could be assumed that the production of resistant variants is not connected with the specific effect of the extract, but depends on the change in the conditions of nutrition of the staphylococcus on medium containing extract. In connection with this, an experi-

Table 2. Effect of the Concentration of Cell-Free Extract on the Transformation of Resistance to Streptomycin in Staphylococcus aureus (duration of experiment 36 hours)

Experiment No.	Dilution of extract									
	—	1 : 2		1 : 5		1 : 10		1 : 100		
	Number of cells									
	Inoculated on plates	Resistant to streptomycin	Inoculated on plates	Resistant to streptomycin	Inoculated on plates	Resistant to streptomycin	Giving one mutation	Inoculated on plates	Resistant to streptomycin	Giving one mutation
1	$2 \cdot 10^7$	0	$12 \cdot 10^7$	0	*	259	—	$2 \cdot 10^7$	0	0
2	$70 \cdot 10^7$	0	—	—	*	1373	—	$40 \cdot 10^7$	3070	$0,19 \cdot 10^7$
3		—	—	—	$115.92 \cdot 10^7$	44 277	$0,2 \cdot 10^5$	$400.9 \cdot 10^7$	30	$0.13 \cdot 10^9$
4		—	—	—	$124.8 \cdot 10^7$	9289	$0,14 \cdot 10^6$	—	—	—

* There was no growth when plated on MPA without streptomycin.

Table 3. The Effect on Transformation of Prolonged Keeping of the Staphylococcus in MPB Containing Cell-Free Extract

Expt. No.	Duration of experiment in hours	Extract dilution				
		1 : 10			1 : 100	
		Number of cells				
		Inoculated on plates	Resistant to streptomycin	Giving one mutation	Inoculated on plates	Resistant to streptomycin
1	5	$76 \cdot 10^3$	1	$0.76 \cdot 10^8$	$72 \cdot 10^6$	0
	18	$364 \cdot 10^3$	413	$0.88 \cdot 10^3$	$353 \cdot 10^6$	0
	26	$68 \cdot 10^3$	498	$0.14 \cdot 10^3$	$455.4 \cdot 10^6$	0
	36	453·750	1000	$0.45 \cdot 10^2$	—	—
2	5	$70 \cdot 10^6$	1	$0.70 \cdot 10^8$	—	—
	18	$66 \cdot 10^6$	10 800	$0.61 \cdot 10^4$	—	—
	26	250·103	15 038	$0.16 \cdot 10^2$	—	—

Table 4. The Effect on Staphylococcus Cells of Cell-Free Extract Obtained From the Original (Unadapted to Streptomycin) Culture

Expt. No.	Duration of experiment in hours	Extract added in 1:10 dilution			Without addition of cell extract		
		Number of cells					
		Inoculated on plates	Resistant to streptomycin	Giving one mutation	Inoculated on plates	Resistant to strepto-mycin	Giving one mutation
1	5	$26 \cdot 10^6$	1	$26 \cdot 10^8$	$26 \cdot 10^6$	0	0
	18	$856 \cdot 10^6$	0	0	$92 \cdot 10^6$	9	$0.1 \cdot 10^9$
	26	$1340 \cdot 10^6$	12	$0,11 \cdot 10^9$	$950 \cdot 10^6$	9	$0.1 \cdot 10^9$
	36	$897 \cdot 10^5$	16	$0.56 \cdot 10^8$	$866 \cdot 10^6$	12	$0.72 \cdot 10^8$

Table 5. The Effect of Cell-Free Extract on the Growth of the Original Staphylococcus Culture

Expt. No.	Duration of experiment in hours	Cell-free extract obtained from original (unadapted to streptomycin) culture (number of cells per ml)		Cell-free extract obtained from culture adapted to streptomycin (number of cells per ml)	
		Dilution 1:10	Without addition of extract	Dilution 1:100	Dilution 1:10
1	5	$26 \cdot 10^5$	$26 \cdot 10^6$	$36 \cdot 10^6$	$38\,000 \cdot 10^3$
	18	$188 \cdot 10^6$	$206 \cdot 10^6$	$141 \cdot 10^6$	$182 \cdot 10^3$
	26	$134 \cdot 10^6$	$190 \cdot 10^6$	$124 \cdot 10^6$	$34 \cdot 10^3$
	36	$138 \cdot 10^6$	$146 \cdot 10^6$	$151.8 \cdot 10^6$	$151.25 \cdot 10^3$
1	5	—	—	—	$29\,000 \cdot 10^3$
	18	—	—	—	$13\,200 \cdot 10^3$
	26	—	—	—	$50 \cdot 10^3$

ment was conducted in which cells of the original staphylococcus culture grew in medium containing cell-free extract obtained from the cells of the original staphylococcus culture. In the control flasks, growth of the staphylococcus took place on medium containing no extract at all. As seen from the data in Table 4, the cell extract of the nonresistant original culture did not cause transformation. The number of resistant variants formed in the medium with extract was the same as in the control, i.e., in medium without extract. Consequently, transformation can not be explained by a change in the conditions of nutrition of the staphylococcus when cell-free extract containing proteins, nucleic acids, and carbohydrates is added. This effect is specific, since it was observed only after the addition of extract from the cells of the resistant variant.

In the course of the work, it was striking that the addition to the medium of extract obtained from the cells of the adapted variant led to the sharp inhibition of growth of the staphylococcus. For the purpose of obtaining more precise data, experiments were set up according to the following plan. The original staphylococcus culture was inoculated: 1) into medium containing extract obtained from the cells of the unadapted original culture in the amount of 1:10; 2) into medium containing no extract at all; 3) into medium to which extract of cells of the adapted variant was added in a concentration of 1:100; 4) into medium containing the same extract, but in a concentration of 1:10. The results of these experiments are given in Table 5. We see that a sharp decrease in the number of cells in the culture was observed only in those flasks to which extract obtained from the cells of the resistant variant was added. In all other cases, normal reproduction of the staphylococcus occurred. The reason for the bacteriostatic effect of the extract cannot be explained by the fact that it becomes toxic in the process of its production, since extract obtained by the same method

Table 6. The Effect of Desoxyribonuclease on the Transformation Phenomenon

Expt. No.	Amount of Desoxyribonuclease, γ per ml	Number of streptomycin-resistant cells per ml	Number of cells giving one mutation
1	30	99	$0.7 \cdot 10^3$
	60	10	$0.2 \cdot 10^5$
2	80	0	0
	0	1239	$0.45 \cdot 10^3$
3	80	10	$0.15 \cdot 10^4$
	0	343	$0.4 \cdot 10^2$

from the cells of the original culture and also added to the medium in a concentration of 1:10 does not inhibit reproduction. It has sometimes been proposed that transformation is brought about not by DNA alone, but by the protein impurities of the DNA as well. In bringing about transformation by means of extracts, it was of interest to treat the extracts with the enzyme, desoxyribonuclease, which decomposes DNA. In connection with this, extract obtained from the cells of the streptomycin-resistant staphylococcus was treated with various amounts of desoxyribonuclease. The data of these experiments, which are given in Table 6, make it possible to conclude that desoxyribonuclease deprives the extract of the ability to cause transformation. At a concentration of 80 γ of desoxyribonuclease per 1 ml, no resistant variants appeared at all in one experiment, while in another, their number decreased 34-fold. These data enable one to assume that the DNA contained in the extract is responsible for transformation. However, more detailed investigations are required for the solution of the problem of the participation of proteins in the phenomenon of transformation.

SUMMARY

1. A study was carried out on an experimentally adapted streptomycin-resistant strain of Staphylococcus aureus. A noncellular but DNA-containing extract was obtained through disintegration of bacterial cells followed by centrifugation.

2. By adding the extract to the meat-peptone broth in which the streptomycin-unadapted staphylococcus culture is grown, it is possible to obtain streptomycin-resistant variants from the original culture.

3. The transformation phenomenon was particularly frequent with a 1:10 dilution of the extract. Less regular was transformation with 1:2, 1:5, and 1:100 dilutions.

4. The original streptomycin-unadapted staphylococcus culture contains only one cell per $0.25 \cdot 10^9$–$0.65 \cdot 10^9$ of the variant resistant to 40,000 units of streptomycin per 1 ml of nutrient medium. Through the effect of extract obtained from the cells of the resistant staphylococcus variant, the number of resistant variants in the culture increased 1000–100,000 times. However, no essentially new variants arise through the effect of extract; only their number rises sharply.

5. The effect of the extract is specific, since extract obtained from the cells of the original nonadapted variant does not exhibit any transformation effect.

6. The transformation effect is apparently linked with the DNA of the extract, since when treated with desoxyribonuclease it loses its transforming capacity.

7. A certain time interval is necessary for the manifestation of the transformation effect. Plating from five-hour staphylococcus cultures growing in extract-containing medium gives almost no resistant variants. Only after 18–26 hours of cultivation does the number of resistant variants produced increase significantly.

8. A 1:10 concentration of extract obtained from the cells of the resistant strain greatly inhibits the development of the culture, while a 1:100 dilution as well as extract obtained from the cells of the original nonresistant strain do not exhibit any effect.

9. It is possible that, under conditions of joint cultivation of two cultures, variability may be observed which is connected with the exchange of DNA released during cell autolysis and exerting a reciprocal transforming effect.

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DESOXYRIBONUCLEIC ACID IN THE MYCELIUM OF ACTINOMYCES AUREOFACIENS STRAIN LS-112 UNDER CONDITIONS OF SUBMERGED CULTIVATION

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Investigations of nucleic acids during the course of growth of antibiotic producers of actinomycete origin such as Actinomyces aureofaciens, Actinomyces streptomycini, and Actinomyces rimosus have been carried out with the aid of both cytological and biochemical methods (Guberniev, Ugoleva, and Torbochkina, 1956a, b; Demyanovskaya and Belozerskii, 1954; Prokof'eva-Bel'govskaya and Demyanovskaya, 1957; Biffi-Gentili, Boretti et al., 1954; Doskočil et al., 1958). In the works of some authors (Guberniev, Ugoleva, and Torbochkina, 1956a; Demyanovskaya and Belozerskii, 1954; Prokof'eva-Bel'govskaya and Demyanovskaya, 1957), the fact that DNA is absent from the mycelium at the early stages of development of the culture (after 3–6 hours following inoculation of the medium) has been noted.

In the present work, we have set ourselves the task of checking our previously obtained data (1956a) concerning the absence of DNA at the early stages of growth of A. aureofaciens.

MATERIALS AND METHODS

Strain LS-112 of A. aureofaciens was used for the investigation. Spores stored on millet or seed mycelium grown for 24 hours on standard corn medium were used as inoculum. The spores or seed mycelium were inoculated into corn medium or into meat-peptone broth with starch (2.5%). Fermentation was carried out both in 45-liter fermentors in the semiproduction plant of the All-Union Scientific Research Institute of Antibiotics and under laboratory conditions. Samples were collected after 5, 10, 12, 24, and 48 hours following inoculation of the medium.

For microscopic examination, preparations were fixed with Carnoy fluid. To demonstrate basophilia of the protoplasm (determined by its RNA content), fixed preparations were stained with methylene blue (0.1% aqueous solution), methyl green-pyronine (according to Brachet), and gallocyaninchrome alum (according to Einarson). Parallel preparations were previously treated with a ribonuclease solution (1 mg/ml) which the authors obtained from the pancreas of cattle by Kunitz' method. In order to demonstrate nuclear elements and their DNA content, fixed preparations were stained with Giemsa solution after hydrolysis in 1 N HCl for seven minutes at 60° (Robinow method), and then staining was completed with light green (according to Peshkov) or the preparations were treated according to Feulgen.

Air-dried mycelium was used for the biochemical investigation. Nucleic acids were extracted according to Schneider's method. Total nucleic acids were determined by phosphorus, according to the method of Fiske and SubbaRow, DNA—by Diesche's method and according to Webb, RNA—by difference.

EXPERIMENTAL RESULTS

In the first series of experiments, we investigated the nucleic acid content during the course of development of the A. aureofaciens culture on corn medium under the conditions of the semiproduction plant at the VNIIA. Seed mycelium was used as the inoculum. The maximal content of RNA was noted in 24-hour mycelium, which corresponds to the stage of intensive growth of mycelium with homogeneous, basophilic protoplasm, rich in RNA (Figs. 1, 2). After 24 hours, the RNA content of the protoplasm decreased. The culture reached a developmental phase in which growth was slowed down and maximal antibiotic production occurred. The hyphal protoplasm began to differentiate (Fig. 3). The quantitative DNA content was more or

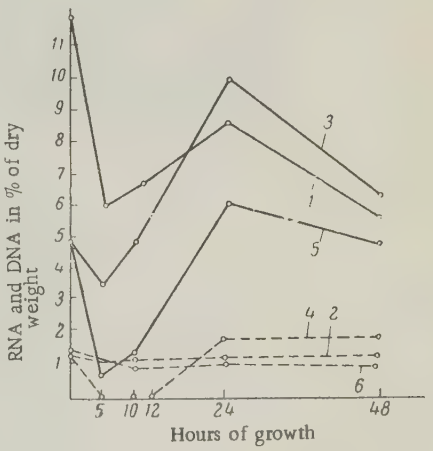

Fig. 1. Nucleic acid content of Actinomyces aureofaciens mycelium at various stages of development. 1) RNA and 2) DNA, on standard medium when inoculated with mycelium; 3) RNA and 4) DNA, on standard medium when inoculated with spores; 5) RNA and 6) DNA, on MPB when inoculated with spores.

DNA Content of Actinomyces aureofaciens Mycelium at Various Hours of Fermentation When the Medium is Inoculated with Spores (in % of dry weight)

Hours of growth	Amount of inoculum	
	optimal	10× inc.
Spores	1.40	1.40
5	0.00	0.97
10	0.00	1.00
15	0.00	1.12
20	Traces	1.68
24	1.64	1.68
48	1.93	1.83

less constant at all stages of development of the actinomycete, but increased somewhat by the 48th hour of fermentation, i.e., at the time of maximal differentiation of the protoplasm (Fig. 1). This was confirmed by cytological data as well: when stained with Giemsa solution after acid hydrolysis, discrete nuclear elements were found at all stages of development. Weak (at early stages) or distinct (at later stages) pinkish staining of the nuclear elements when using Feulgen's method is evidence of the presence of DNA in them. The behavior of nuclear elements in the early stages of development (Figs. 4, 5) is of particular interest, since in our preceding investigation, DNA was not found during this period (1956a). In this case, the culture apparently grew more slowly than in the experiments described, and after five hours following inoculation of the medium, only the start of sprouting of the seed mycelium was observed. During this period, the main mass of mycelium evidently consisted of young sprouts, the nuclear elements of which are small and contain small amounts of DNA which could be detected neither by biochemical (Diesche method) nor by cytochemical methods (Feulgen reaction). In the present investigation, two chemical methods of determining DNA (the Diesche method and the more sensitive Webb method) were used simultaneously, which enabled us to detect even small amounts of DNA.

In the second series of experiments (Fig. 1), we investigated the nucleic acid content during growth of the culture on the same medium under laboratory conditions. Spores of the same strain were used as inoculum. This gave us the opportunity to obtain more uniform material at the early stages of development. As in the first series of experiments, maximal RNA content was observed in 24-hour mycelium (Fig. 1), which corresponds to the phase of intensive growth of the mycelium with homogeneous basophilic protoplasm, rich in RNA. No DNA was found by biochemical methods in 5- and 10-hour samples. Microscopic analysis showed that these hours of fermentation corresponded to the earlier (five hours) or later (ten hours) stages of spore germination. When stained according to Robinow's method, distinct nuclear elements (Figs. 6, 7) were found in germinating spores and in young sprouts developing from spores. The fact that they stained pinkish-purple by Feulgen's method is evidence of the presence of DNA in them. The fact that DNA is absent at the indicated stages of development according to the data of biochemical investigations is apparently explained by the extremely small biomass in the presence of a large amount of impuri-

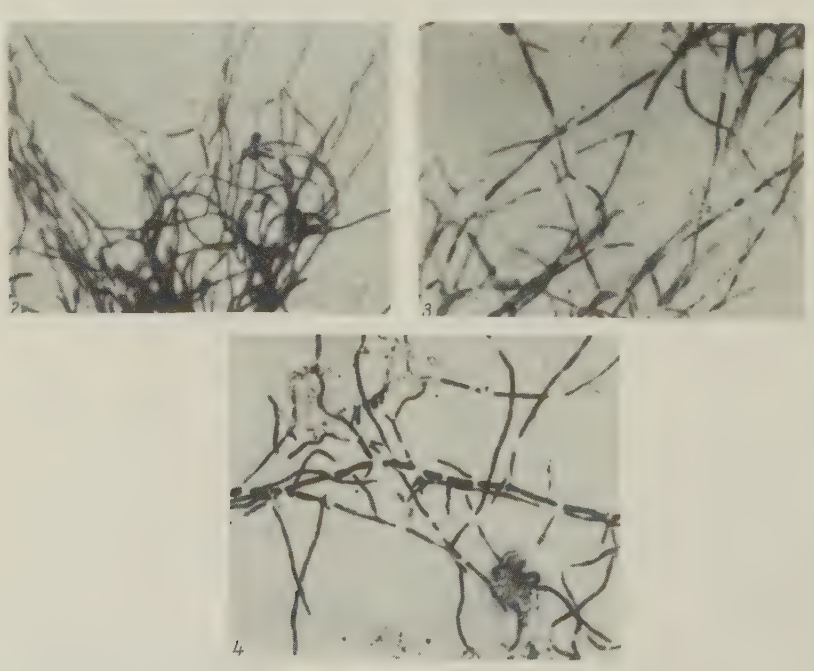

Fig. 2-7. Nuclear elements in mycelium which had developed from spores and from seed mycelium.

Fig. 2. Twenty-four hours of growth. Methylene blue stain. Magnification 2000 × (15 × 90).

Fig. 3. Forty-eight hours of growth. Methylene blue stain. Magnification 2000 ×.

Fig. 4. Five hours, when medium was inoculated with seed mycelium. Methylene blue stain. Magnification 2000 ×.

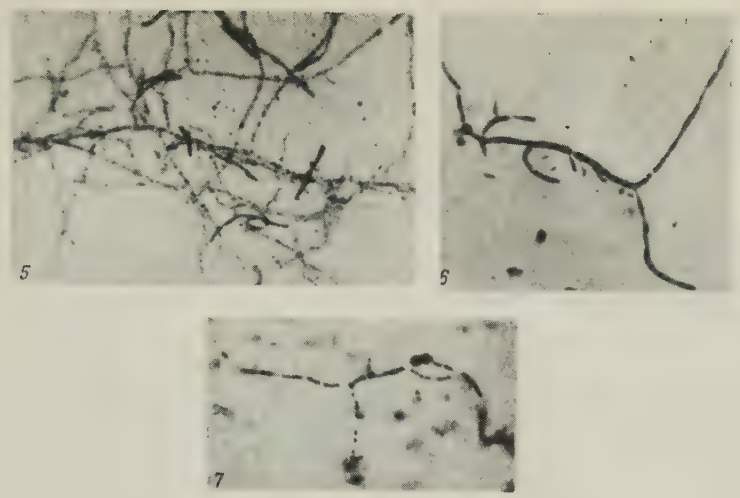

Fig. 5. Five hours, when inoculated with seed mycelium. Giemsa–light green stain. Magnification 1600 x (15 x 90).

Fig. 6. Ten hours of growth when inoculated with spores. Methylene blue stain. Magnification 2000 x.

Fig. 7. Ten hours when inoculated with spores. Giemsa–light green stain. Magnification 2000 x.

ties in the medium. Microscopic analysis of the dry material used for the biochemical determinations of nucleic acid confirmed this hypothesis.

In especially designed experiments, where the amount of spores added to the medium was increased tenfold, it was possible to detect DNA in the early stages by biochemical methods as well (Table). Microscopic examination showed that the majority of spores germinated normally under these conditions. Treatment of the preparations according to Feulgen confirmed the biochemical data concerning the presence of DNA in germinating spores.

In the third series of experiments (Fig. 1), in order to eliminate the impurities in the medium, we used a relatively transparent medium (meat-peptone broth) as the fermentation medium. Spores were used as inoculum. On this medium, spore germination was retarded, so that the biomass was extremely small even by the tenth hour of fermentation. At the same time, DNA was detected in the mycelium at the early stages of development not only by cytochemical, but by biochemical methods as well.

Although the development of the culture on meat-peptone broth and on corn medium proceeded differently, the behavior of nuclear elements in germinating spores was similar. Thus, the data obtained in the last series of experiments make it reasonable to suppose that there is a possible difficulty in determining DNA in mycelium quantitatively at the early stages of development when complex media are used.

SUMMARY

1. When Actinomyces aureofaciens, strain LS-112, is grown on corn medium and on meat-peptone broth, there are discrete nuclear elements in the mycelium at all stages of development.

2. Nuclear elements are found in the mycelium developing both from the germination of spores and the germination of seed mycelium.

3. DNA is found in the nuclear elements of A. aureofaciens, strain LS-112, at all stages of development.

4. However, in the early stages of development in complex media, the determination of DNA by biochemical methods is difficult due to the extremely small biomass and the large amount of unused components of the medium.

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THE EFFECT OF TEMPERATURE ON THE NUCLEIC ACIDS OF ASPERGILLUS FUMIGATUS

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The given work is a continuation of our investigation of the effect of temperature on the nucleic acids of lower organisms growing at high and ordinary temperatures (Oparin and Evreinova, 1947). Temperature is regarded as an energy factor and an accelerator of enzymatic reactions. It was shown in preceding works that when the temperature of the surrounding medium is increased, the content of purine and pyrimidine bases (Evreinova, Ermolaeva, and Gerasimova, 1958; Evreinova, Bova, and Rzhanova, 1958) and nucleic acids in bacteria decreases, and the mononucleotide composition of ribonucleic acid also changes (Evreinova, Bunina, and Kuznetsova, 1959).

In the given work, the content and mononucleotide composition of the nucleic acids in a thermophilic and a mesophilic variant of Aspergillus fumigatus, and also proteins, were determined.

No data have been found in the literature on the comparative study of nucleic acids as a function of different growth temperatures of a given species of fungus.

TEMPERATURE ADAPTATION OF ASPERGILLUS FUMIGATUS

Variants growing at 48–50° (thermophils) and at 23–25° (mesophils) were isolated from the original 2/I strain of A. fumigatus growing at 30–35° by means of numerous consecutive transfers over a long period of time while constantly increasing and decreasing the temperature.

Adaptation experiments were conducted with the fungus growing on Petri dishes of Czapek's medium of the following composition: $NaNO_3$—3 g, KH_2PO_4—1 g, $MgSO_4$—0.5 g, KCl—0.5 g, $FeSO_4$—10 mg, 20 g of Archangel agar, and 20 g of glucose per 1 liter of tap water as the carbon source. The medium was sterilized for 30 minutes at 0.5 atm. The medium was inoculated with fungal spores under sterile conditions. Sporulation of the fungus was normal both at 25° and at 50°. The sporulation of the thermophilic culture is especially important, because increasing the temperature frequently leads to the loss of sporulation. The mycelium of both cultures grew well; in the thermophils, growth was considerably faster. For example, a 38–40-hour culture of thermophils had a completely developed mycelium with spores, while the mesophils reached this state only after 50 hours of growth. For analyses, the cultures were grown by the submerged method on shakers; they were incubated at 23–25 and 48–50° in the dark in order to exclude the additional effect of light (Tatarenko, 1954). Inoculations were made under sterile conditions with spores of the appropriate cultures obtained by growing the fungus by the surface method. Two billion spores were inoculated into 200 ml of liquid medium. The composition of the medium was essentially the same as in the case of surface cultivation with the exception of the following: absence of agar, replacement of glucose by the same amount of starch, and the addition of 5 g of corn extract which speeded up the growth of the fungus considerably (conditions of sterilization of the medium: 30 minutes, 0.5 atm).

In order to obtain comparative data, it was necessary to have thermophilic and mesophilic cultures of the fungus taken at the same stages of development. For this purpose, we used Bekker's directions (1956), who established the sequence of the different phases in the development of a number of fungi including that of A. fumigatus. After analyzing all of the phases of our cultures, the third phase, characterized by a maximal content of nucleic acids and lipids and by the best mycelial growth, was used for accumulating a mass. In thermophils, this phase was reached in 24 hours, while in the mesophils—in 40 hours of growth. At the end of the cultivation time, the mycelium was separated from the culture medium, was washed with water, and was then dehydrated by freezing and lyophil drying. Nucleic acids as well as various forms of organic nitrogen were studied in this material. The different forms of organic nitrogen served as an indicator of the state of the culture.

Forms of nitrogen. Total nitrogen as well as the protein and nonprotein nitrogen fraction obtained by Barnstein's method were determined according to Kjeldahl with distillation of ammonia by the semimicro method (Belozerskii and Proskurakov, 1951).

The results of the analyses are given in Table 1.

Table 1. Forms of Nitrogen in Aspergillus fumigatus Cultures (in % of dry weight)

Culture	Nitrogen		
	non-protein	protein *	Total
Mesophils	0.98	4.68	5.58
Thermophils	0.35	4.38	4.74

* The protein nitrogen fraction included the nitrogen of proteins, nucleic acids, and lipoproteins.

The data in Table 1 show that autolysis was practically absent in the fungal cultures, as evidenced by the low percentage of nonprotein nitrogen. This is of significance in the given case, because there are active proteases in A. fumigatus. The major portion of the total nitrogen was accounted for by high-molecular substances. There was hardly any quantitative change in the latter with temperature.

NUCLEIC ACIDS

METHODS

The method of determining nucleic acids—ribonucleic (RNA) and desoxyribonucleic (DNA)—as well as formulas and calculations have been set forth in detail in our communication (Evreinova, Bunina, and Kuznetsova, 1959). This method was first proposed by Schmidt and Tannhauser, and then modified by a number of authors (Lesley, 1957).

Low-molecular substances including mononucleotides were removed from 600 mg of dry mycelial mass. The residue was hydrolyzed with alkali. By the end of hydrolysis, the RNA breaks down to mononucleotides and in this form is isolated from the fraction containing the DNA. The latter is hydrolyzed with $HClO_4$ to purine and pyrimidine bases. Following treatment, the RNA and DNA hydrolyzates are placed on paper chromatograms and are fractionated into individual compounds by means of the appropriate solvents.

Figures 1 and 2 are photographs of chromatograms of RNA mononucleotides taken in ultraviolet light (Evreinova and Ermolaeva, 1958) after successive runs first in solvent I containing ethanol and butanol, and then in solvent II consisting of isobutyric acid. Mononucleotides were eluted from the chromatograms with 1 M phosphate buffer at pH 7, while purine and pyrimidine bases were eluted with a 1/10 N solution of HCl. The concentration of the substances in the eluates was measured spectrophotometrically on an SF-4 spectrophotometer.

In determining RNA by this method, the concentration of the alkali used for hydrolysis is very important. Ordinarily, from 0.1 to 1 N KOH is used depending on the material (Lesley, 1957). The most widely used is 0.5 N KOH (Spirin, 1957). We used from 0.3 to 1/1 N alkali. The best results were obtained when the mass was hydrolyzed with 0.8 N KOH for 18 hours at 37°. In this case, 4–5 ml of 0.8 N KOH was used for all of the material.

Cytidylic acid is converted to uridylic by the action of strong alkalis; in our experiments, however, where hydrolysis was carried out with 0.8 N KOH, cytidylic acid was not broken down. The yield of all mononucleotides of both the thermophilic and mesophilic cultures was 1.5–1.7 times higher when hydrolysis was carried out with 0.8 N KOH than when it was carried out with 0.5 N KOH, and the greatest increase was due to purine mononucleotides: for example, the amount of adenylic and guanylic acid in the thermophilic variant rose twofold, while in the mesophilic—1.8-fold. Therefore, data obtained using hydrolysis with 0.8 N KOH are presented in the tables. An "excess" of alkali is evidently used for the hydrolytic

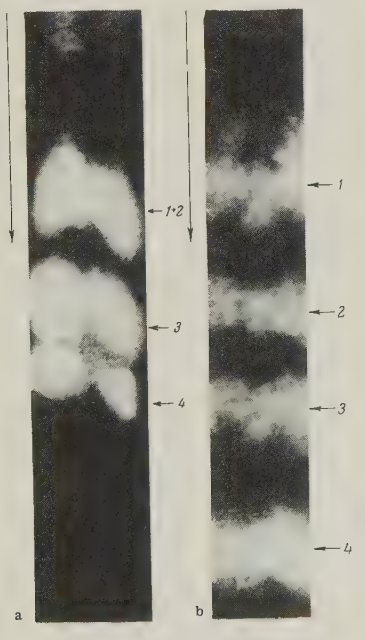

Fig. 1.

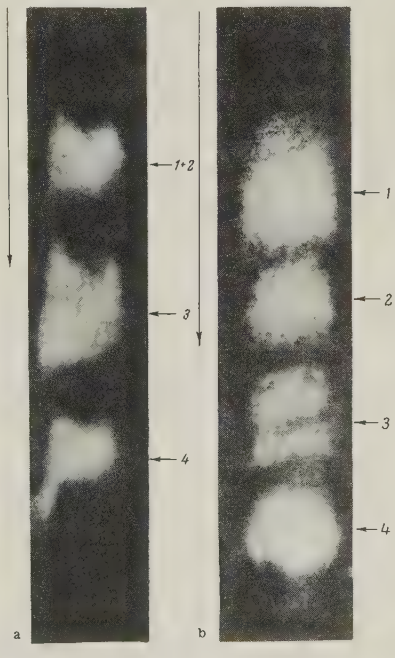

Fig. 2.

Fig. 1. Chromatograms of RNA mononucleotides. Mesophilic culture. a) Fractionation in solvent I; b) fractionation in solvent II.

Fig. 2. Chromatograms of RNA mononucleotides. Thermophilic culture. a) Fractionation in solvent I; b) fractionation in solvent II.

Designation of spots on chromatograms (from the top down). 1) Guanylic acid; 2) uridylic acid; 3) cytidylic acid; 4) adenylic acid.

Table 2. The Mononucleotide Composition of RNA in Per Cent of Dry Weight and in Molar Per Cent (figures in parentheses)*

Mononucleotides	Culture	
	Mesophils	Thermophils
Guanylic acid	0.82(29.58)	0.57(30.15)
Uridylic "	0.48(19.20)	0.37(21.69)
Cytidylic "	0.58(24.00)	0.38(22.14)
Adenylic "	0.72(27.22)	0.49(26.02)

*Molar percent is the ratio of the number of molecules of the given mononucleotide to the sum of all mononucleotides, expressed in percent.

Table 3. The Composition of RNA According to Chargaff's Rules (Belozerskii and Spirin, 1956)

Culture	Nativeness $\frac{G+U*}{A+C}$	Purines / pyrimidines	Type $\frac{G+C}{A+U}$
Mesophils	0.96	1.31	1.13
Thermophils	1.07	1.29	1.09

*A—adenylic acid; G—guanylic; U—uridylic; C—cytidylic.

splitting of other compounds to which RNA is bound (Venner, 1958).

The concentration of perchloric acid is very important in the hydrolysis of the DNA fraction. Ordinarily, 72% $HClO_4$ is the most frequently used. In our work, hydrolysis was carried out both with 72% and with 59% $HClO_4$ for an hour on a boiling water bath. In both cases, 0.2 ml of $HClO_4$ was used for 10 mg of fraction. Analyses showed that the total of all bases was eight times greater in the case of a hydrolysis with 59% $HClO_4$ than with 72% acid. The figures obtained for 59% $HClO_4$ are given in Tables 4 and 5.

In Tables 2 and 3, data are given on the mononucleotide composition of RNA, while in Tables 4 and 5—the mononucleotide composition of DNA.

Table 4. The Mononucleotide Composition of DNA in % of Dry Weight of Mycelium and in Molar Per Cent (figures in parentheses)*

Mononucleotides	Culture	
	Mesophils	Thermophils
Desoxyguanylic acid	0.23(27.14)	0.064(26.69)
Desoxyadenylic acid	0.19(25.09)	0.061(26.27)
Desoxycytidylic acid	0.18(23.58)	0.052(23.17)
Desoxythymidylic acid	0.19(24.19)	0.071(23.87)

* DNA, similarly to RNA, is built of mononucleotides; therefore, in this table, the listed values are given (from purine and pyrimidine bases measured directly) for the corresponding mononucleotides.

Table 5. Composition of DNA According to Chargaff's Rules

Culture	Nativeness $\frac{G+T*}{A+C}$	Purines / pyrimidines	Type $\frac{G+C*}{A+T}$
Mesophils	1.056	1.037	1.095
Thermophils	1.023	0.995	1.128

*T—desoxythymidylic acid; the rest of the designations are the same as in Table 3, except that desoxyribose instead of ribose is present in all mononucleotides.

It is seen from the data in Tables 2 and 3 that, as the growth temperature of the fungus was increased, the amount of RNA nucleotides decreased and that its nucleotide composition changed as well. The RNA of the mesophilic and thermophilic variants of A. fumigatus belonged to the weakly-expressed G+C type in which the sum of guanylic and cytidylic acids predominates slightly over the sum of adenylic and uridylic acids, especially in the thermophils. Similar to our data on the type of RNA in the mesophilic variant was that obtained by Kulaev for Aspergillus niger (Kulaev, 1956).

Table 6. Number of Molecules of RNA and DNA Mononucleotides Per 1 g of Dry Weight in Aspergillus fumigatus Cultures

Mononucleotide	Absolute weight of molecule $\times 10^{-16}$	No. of molecules $\times 10^6$ Culture	
		Mesophils	Thermophils
Ribonucleic acid			
Guanylic acid	5.81	80 000	46 000
Adenylic "	5.55	77 000	43 000
Cytidylic "	5.17	73 000	48 000
Uridylic "	5.18	69 000	46 000
Total		299 000	183 000
Desoxyribonucleic acid			
Desoxyguanylic acid	5.40	42 000	42 000
Desoxyadenylic "	5.12	37 000	12 000
Desoxycytidylic "	4.72	40 000	9 000
Desoxythymidylic "	4.99	38 000	14 000
Total		157 000	47 000

The data in Tables 4 and 5 show that an increase in the growth temperature was accompanied by a decrease in the amount of mononucleotides comprising the DNA of A. fumigatus. The mononucleotide composition of DNA was practically the same in both thermophilic and mesophilic cultures. The DNA of these cultures belongs to the G+C type, close to unity, in which the sum of the desoxyguanylic and desoxycytidylic acids exceeds the sum of desoxyadenylic and desoxythymidylic acids very slightly.

Since molecules take part in the reactions occurring in microorganisms, we felt it to be of interest to imagine the number of RNA and DNA mononucleotide molecules which can be used by the fungus. Results of the mathematical treatment are given in Table 6.

It is seen from the data in Table 6 that tens of billions of mononucleotide molecules are present in the nucleic acids of the mesophilic and thermophilic cultures of A. fumigatus which can be utilized by the fungus for synthesizing enzyme systems.

The percent of RNA and DNA in A. fumigatus cultures is given in Fig. 3. In this case, the percent of nucleic acids is calculated on the basis of the sum of the mononucleotides comprising them.

It is seen from Fig. 3 that an increase in temperature led to the decrease of the content of both RNA and DNA. In the mesophilic culture, 26.25% protein was found, while in the thermophilic—25.67% (protein was calculated by difference between total protein determined by protein nitrogen and the sum of nucleic acids). Thus, the thermophilic and mesophilic cultures of the fungus differ insignificantly from one another in protein content.

DISCUSSION OF RESULTS

With a rise in growth temperature of the fungus A. fumigatus, taken at identical stages of development and containing similar amounts of protein in the mycelium, a decrease in the per cent of both RNA and DNA takes place. We obtained analogous data for the thermophilic and mesophilic variants of Bacillus licheniformis (Evreinova, 1959). There are similar indications with the effect of radiations on organisms as well (Bacq, 1955).

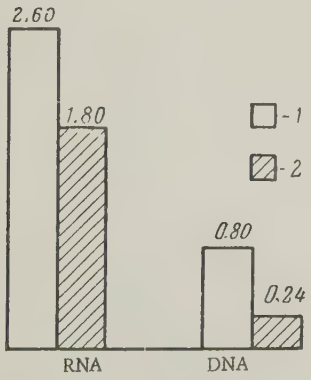

Fig. 3. Content of RNA and DNA in per cent of dry weight of the cultures. 1) Mesophils; 2) thermophils.

Aside from this, the temperature has an effect on the mononucleotide composition of the RNA of the thermophilic variant of A. fumigatus. The same thing was also observed in the thermophilic culture of B. licheniformis. According to the indications available in the literature, the mononucleotide composition of RNA remains unchanged within the bounds of the species (Belozerskii, 1957); however, data obtained in studies of the effect of various nutritional factors and alterations due to age, but not the effect of temperature, served for these conclusions. Recently, it has been shown in the work of Brawerman and Chargaff (1959) that the mononucleotide composition of RNA in Euglena gracilis grown in the dark (not photosynthesizing) differs from that in the light (photosynthesizing).

It seems to us that the effect of high temperatures on organisms and the acquiring by them of the ability to live at such temperatures affects the entire energy and enzymatic metabolism, which leads to changes in the mononucleotide composition of RNA, if the latter is regarded as closely associated with this metabolism. Light energy can also be treated as a special supplementary energy obtained by the cell and leading to an entirely different type of metabolism, which again reflects on the nucleotide composition of RNA.

SUMMARY

An increase in the growth temperature of the fungus Aspergillus fumigatus from 25 to 50° is accompanied by a decrease in the amount of nucleic acids and by a change in the nucleotide composition of ribonucleic acids. The composition of DNA remains constant.

The RNA and DNA of A. fumigatus belong to the weakly-expressed G+C type—close to unity.

The nitrogenous organic substances of A. fumigatus consist chiefly of proteins. The content of the latter in the cultures show hardly any dependence on the growth temperature of the fungus.

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A PIGMENTED FORM OF NITROSOMONAS EUROPAEA

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About 70 years ago, in describing the inducer of the first phase of nitrification which he discovered, Nitrosomonas europaea, S. N. Vinogradskii pointed out that there are two morphological forms of this organism: a form growing on solid substrate in the form of an aggregate of cells surrounded by a common slimy mass and resembling zoogloea, and a form represented by free flagellated cells (Vinogradskii, 1952). Thenceforth, the morphology of Nitrosomonas has been studied frequently (Kingma Boltjes, 1935; Imshenetskii, 1945; Lees, 1953 and others). Most recently, a detailed electron microscopic investigation of Nitrosomonas has been carried out by Ruban (1960). The author obtained new data on the morphology of the free cells and observed the microscopic picture of the formation of zoogloea and the production of flagellated forms.

However, if one does not count two directly opposite findings, information on the comparative physiology of zoogloea and free cells is practically absent in the literature. Thus, S. N. Vinogradskii noted that the free cells of nitrite bacteria are more active than cells found in chalk sediment. Ruban and Zavarzin (1955), however, point out that with Peshkov's double stain (Giemsa-light green), "the cells which are basophilic come from clumps of chalk, almost exclusively. Cells which are in the liquid phase, on the other hand, are stained green with light green and are evidently inactive."

In the present communication, we shall dwell on some results of a study of the comparative physiology of zoogloea and of free cells.

The work was carried out with a pure culture of the same strain of nitrite bacteria with which Ruban worked. The Nitrosomonas for the experiments was cultured at the experimental unit of the Institute of Microbiology of the Academy of Sciences, USSR, under the direction of I. V. Ulezlo to whom we extend deep appreciation. The bacteria were cultured in 100 liter fermentors containing 90 liters of Vinogradskii's medium with chalk. Cultivation was carried out for 12–14 days at 28° with slight aeration. After growth, the bacteria and chalk sediment were separated out by filtration through a bacterial membrane filter. The purity of the culture after cultivation was checked by inoculation into meat-peptone broth as well as on a number of other media recommended by Ruban (1955).

By growing Nitrosomonas in a fermentor, it was possible to obtain the bacteria in considerable quantities and to discover certain facts which had earlier escaped the attention of investigators or which had resisted investigation due to the extremely sparse growth of nitrite bacteria.

1. A PIGMENTED FORM OF NITROSOMONAS

After carefully dissolving the chalk and differentially centrifuging the cell suspension, it was possible to obtain two fractions of bacterial cells with markedly different pigmentation: a fraction which had an intense brownish-red color, and a fraction colored light brown or nearly colorless. Microscopy showed that the first of these consisted of single free cells, while the second consisted of cells collected in aggregates—zoogloea.

As seen from the electron photomicrographs presented, the morphology of zoogloea and free cells is in complete agreement with Ruban's data. Photomicrographs of the red form are given in Fig. 1. Single and sometimes dividing cells are clearly seen (Fig. 1). Frequently, but by far not always, organisms are encountered which have a tuft of flagella on one of their poles (Figs. 2 and 3).

An electron photomicrograph of a zoogloeal form devoid of red pigmentation is given in Fig. 4.

Pigmented cells were easily isolated even without dissolving chalk. In this case, the chalk sediment with zoogloea was separated out by centrifugation at low speeds, and then, at higher rotation speeds (3000 rpm), free, red-colored cells were separated out.

All these data, which were checked many times, enable us to conclude that the two morphological forms of Nitrosomonas described by Vinogradskii differ not only in morphological characteristics, but in the presence of pigment as well. The free cells in the liquid phase have an intense red-brown color. The zoogloea which usually grow on particles of chalk or of other solid substrate, on the contrary, are nearly colorless or are colored light brown.

2. THE NATURE OF THE PIGMENT

The nature of the pigment was not specially studied. However, preliminary data show that it is soluble in water, and that it is not extracted by such solvents as benzene, toluene, chloroform, and benzine, i.e., it does not belong to the carotinoids which dissolve well in lipid solvents. On the other hand, both aqueous extracts from disrupted pigmented (red) cells and from intact cells give a very strong positive reaction with benzidine which is characteristic for hematins. Under

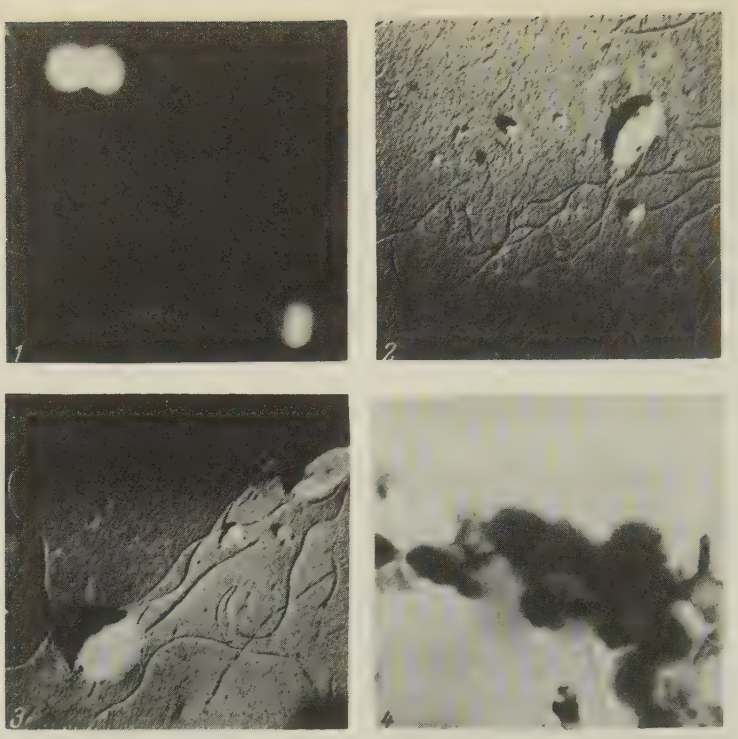

Fig. 1. Free cells, single and dividing. Electron photomicrograph. Magnification $6 \cdot 10^3$x.

Fig. 2. Free cells with flagella. Electron photomicrograph. Magnification $6 \cdot 10^3$x.

Fig. 3. Free cells with flagella. Electron photomicrograph. Magnification $6 \cdot 10^3$x.

Fig. 4. Zoogloea. Electron photomicrograph. Magnification $6 \cdot 10^3$x.

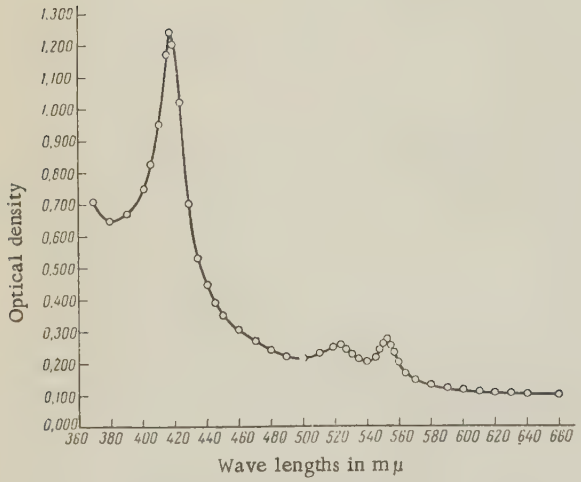

Fig. 5. Absorption spectrum of aqueous extract of disrupted pigmented (red) cells.

similar conditions, although the zoogloeal form of Nitrosomonas which does not have red pigmentation gives a positive reaction with benzidine, it is incomparably weaker. It can thus be assumed that the pigment is of a hematin nature. This assumption finds some confirmation in spectroscopic investigations as well.

3. ABSORPTION SPECTRUM

The absorption spectrum of both forms of Nitrosomonas was studied using both intact cell suspensions and aqueous extracts of cells disrupted with a magnetostriction device.

The results of spectroscopic observations in the visible part of the spectrum (carried out with the aid of a Zeiss spectroscopic microscope attachment) were found to be as follows:

A clearly pronounced absorption band in the region of 550–555 mμ and a somewhat less marked band in the region of 520–525 mμ were observed in suspensions and aqueous extracts of cells having red pigmentation. These bands, which were detectable in the absence of any reducing substances, intensified sharply with the addition of the usual reducing substance for cytochromes—hydrosulfite—and, on the contrary, weakened with aeration.

In suspensions and extracts of cells which did not have red pigmentation, even in the presence of hydrosulfite, a single hardly noticeable band could only occasionally be observed in the region of 550–555 mμ, while in the majority of experiments, no absorption bands at all were noticeable in the visible part of the spectrum.

Spectrophotometric observations carried out with extracts of pigmented cells using an SF-4 spectrophotometer showed that there are three distinct absorption maxima in the wavelength interval from 370 to 660 mμ in the following regions: 418 (Soret region), 522–524, and 552 mμ (Fig. 5). As is well known, these maxima are characteristic for cytochrome C.

Thus, the pigmented cells evidently have a well developed oxidative system, one of the components of

which is cytochrome C. In cells devoid of red pigmentation (zoogloeal form of C. Nitrosomonas), on the contrary, the cytochrome system is much more poorly developed, if present at all.

4. THE PHYSIOLOGICAL ROLE OF CYTOCHROME C

When $(NH_4)_2SO_4$—a substrate which is usually easily oxidized by nitrite bacteria—was added to a suspension of the red cells, neither the appearance or a noticeable intensification of the absorption bands of reduced cytochrome were observed. In the presence of hydroxylamine, which according to all available data is an intermediate product of NH_4^+ oxidation, the bands intensify, but to a considerably lesser extent than with the addition of hydrosulfite. Thus, no clear data were obtained which would indicate the participation of cytochrome C in NH_4^+ oxidation.

It should be noted that in analogous experiments with Nitrobacter, the inducer of the second phase of nitrification, Zavarzin (1958) observed the appearance of distinct absorption bands for reduced cytochromes C and A with the addition of nitrites (which are oxidized to nitrates by this microbe).

Table 1. Intensity of Nitrification and Residual Nitrification in Suspensions of Free (Red) Cells (in mg of NO_2^- per 1 g of cellular organic matter per 1 hr)

Experiment No.	1	2	3	4	5
Intensity of nitrification in medium with $(NH_4)_2SO_4$	24.6	62.0	36.5	90.0	55.2
Residual nitrification	2.6	2.9	1.5	3.0	1.0

Table 2. Production of Nitrites and Residual Nitrification in Suspensions of Zoogloea (in mg of NO_2^- per 1 liter of medium per 1 hr)

Experiment No.	1	2	3
Production of nitrites in medium with $(NH_4)_2SO_4$	3.02	0.86	0.26
Residual nitrification	0.05	0.06	Not determined

However, the absence of noticeable cytochrome reduction when $(NH_4)_2SO_4$ is added and relatively weak reduction in the presence of NH_2OH in our experiments is not sufficient reason to deny the participation of the cytochrome system in NH_4^+ oxidation in Nitrosomonas. It can be supposed, for example, that when NH_4^+ is oxidized, the equilibrium between oxidized and reduced cytochrome is shifted in the direction of the former to such an extent that the experimental methods used did not permit the detection of the absorption bands of the reduced form. It is also possible that neither $(NH_4)_2SO_4$ nor NH_2OH, but rather some other intermediate product in the oxidation of NH_4^+ to NO_2^-, serves as the immediate oxidizable substrate for the cytochrome system. These questions require further study.

5. NITRIFICATION CAPACITY

As has been noted above, the capacity of the free cells and the zoogloeal forms of Nitrosomonas for nitrification remains essentially undetermined. Much physiological work with nitrite bacteria has been carried out without taking the existence of these forms into consideration at all, without separating them, and using the total cell mass present in the culture studied. The accumulation of nitrite bacteria in amounts sufficient for experiments and obtaining both morphological forms in the isolated form made it possible to partially resolve the question of the capacity of these forms for nitrification.

The experiments were set up in 100 ml Erlenmeyer flasks containing 5 ml of medium [0.05 M phosphate buffer at pH 7.8 with 2% $(NH_4)_2SO_4$]. In order to avoid contamination by NH_3 from without, the flasks were sealed with stoppers with sulfuric acid which are usually employed for fermentation studies. Free cells were added to the flasks in the form of a bacterial suspension (containing 30 mg of wet cells per 1 ml), using 0.2 ml per flask. The sediment of zoogloea grown on chalk particles and washed free of free cells was added without dissolving the chalk in order to avoid injuring the cells.

Incubation was carried out for 3–5 hours at 28° with gentle shaking to improve aeration.

In experiments with free cells, the amount of nitrites formed was calculated for 1 mg of organic substance of the bacterial cells. However, this calculation could not be made in experiments with zoogloea, the organic material of which would also include the material of the inert slimy capsule. It also proved methodologically very complicated to use any other indicators of biomass of the cells of the zoogloeal form immersed in slime (for example, number of cells, amount of protein nitrogen, etc.). Therefore, despite the great interest of the comparison of nitrification intensity of the cytochrome-rich pigmented form and the zoogloeal form, we were obliged to limit ourselves for the time being to essentially qualitative data with regard to the latter.

The experimental results are given in Tables 1 and 2. In the lower line of the tables, values are given for so-called residual nitrification (Ruban and Zavarzin, 1955), i.e., the production of nitrites in medium completely free of NH_4^+ due to some internal resources of the cells.

As seen from the tables, both the pigmented free cells of Nitrosomonas and its zoogloeal form which is devoid of red pigmentation actively nitrify $(NH_4)_2SO_4$ and have the capacity for residual nitrification.

The authors express deep appreciation to Prof. A. A. Imshenetskii of the Academy of Sciences, USSR, in whose laboratory and under whose direction the present work was conducted.

SUMMARY

1. The two morphological forms of the Nitrosomonas europaea strain tested (free cells and the zoogloeal form) detected by S. N. Vinogradskii differ not only in morphological characters but also in pigmentation. The zoogloeal form is almost colorless or is light brownish whereas the free cells contain a red-brown pigment.

2. Preliminary data show that the pigment is of a hematin nature.

3. The pigmented Nitrosomonas form exhibits very pronounced adsorption bands characteristic of cytochrome C. In the form devoid of the pigment these bands are very light or are not found at all.

4. Both forms of Nitrosomonas actively nitrify $(NH_4)_2SO_4$ and show the capacity for residual nitrification.

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SYMBIOTIC RELATIONSHIPS BETWEEN CLOSTRIDIUM PASTEURIANUM AND BACILLUS CLOSTEROIDES

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One of the most interesting features of the physiology of Clostridium pasteurianum is that in nature it is always found associated with aerobic sporeforming bacteria (Vinogradskii, 1952; Isachenko, 1951; Omelyanskii, 1953; and others).

We should note that none of the above-mentioned authors regarded the associates of C. pasteurianum as symbionts of it. Hence, considerable interest is provided by Rabotnova's investigation, 1952, the results of which revealed for the first time that the relationships between C. pasteurianum and Bacillus closteroides were symbiotic.

When cultures of C. pasteurianum were isolated by Vinogradskii's method, Rabotnova found only one spore-forming associate, which she identified as B. closteroides. This associate organism was also isolated from C. pasteurianum cultures obtained from a single colony. It was found that under the microscope it was difficult to distinguish cells of the associate from cells of C. pasteurianum owing to their morphological similarity, and that the most reliable method of revealing the associate in cultures of C. pasteurianum was plating out on MPA. B. closteroides, which Rabotnova discovered in cultures of C. pasteurianum, had previously been found by all the authors who had worked with C. pasteurianum, but it had been described under different names.

In our research on the biology and ecology of C. pasteurianum, we discovered a number of facts regarding its associate B. closteroides, and we give an account of these below.

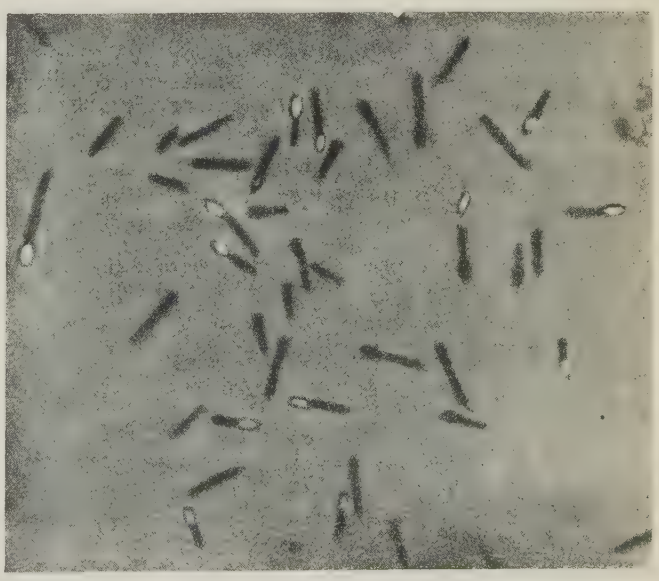
ISOLATION OF BACILLUS CLOSTEROIDES AND A STUDY OF ITS MORPHOLOGY AND PHYSIOLOGY

In our investigation of C. pasteurianum we isolated enrichment cultures, which provided the initial material for the obtention of pure cultures of C. pasteurianum.

The enrichment cultures of C. pasteurianum were obtained by Vinogradskii's method. We isolated 35 enrichment cultures of C. pasteurianum from the soil of the rhizosphere and roots of several crop plants growing on cultivated moderately podzolized turfed loams in Moscow Oblast (Scientific Research Institute for Agriculture in the Central Regions of the Non-Chernozem Zone, Nemchinovka Station, Kalinin Railway).

To keep the cultures in an active state we subcultured them once a month, and in every case the inoculum was pasteurized at 80° for 15 min. As a result of this pasteurization all the concomitant nonsporing microflora perished, and after three to six subcultures the enrichment cultures of C. pasteurianum contained only one sporeforming organism, which grew well on MPA. In almost every case we obtained a pure culture of the associate from the enrichment cultures of C. pasteurianum by plating out on MPA. The morphology and physiology of 13 strains of the associate (L12, Ya16, O18, K21, O15, Ya19, Ya20, Ya24, Ya30, G34, Ts39, Ya25, and K32) were studied. Since no significant differences were found in the morphological, cultural and physiological properties of the individual strains of the associate organism, we give a general description of the associate.

A 48-hr culture of the associate on MPA consisted of motile rods of dimensions $4.0-6.0 \times 0.9-1.1\mu$, with spores situated subterminally or centrally (Figure).



A 3-day-old culture of Bacillus closteroides strain O18 on MPA. Mag. 1600×.

The spores were almost cylindrical, with rounded ends. The cells were slightly inflated and resembled Clostridium; most of the cells were club-shaped. They were Gram-positive. On MPA they formed flat round colo-

nies with ribbed margins, greyish white in color and of mucous consistency. The colonies had a pale center and a marginal ring. In growth on meat-peptone broth a pellicle and turbidity appeared at first, and then a flocculent precipitate was formed. On potato the bacterium gave a fluffy growth of grey colonies. It did not liquefy gelatin. Litmus milk became red and was slowly peptonized. Nitrates were reduced to nitrites. It fermented sucrose, maltose, glucose, and lactose, with the formation of acids. It did not produce gas.

On media containing peptone, asparagine, glycine, leucine or glutamine as the only source of nitrogen the associate microorganism grew very weakly. The addition of glucose to these media did not improve the growth. On mineral sources of nitrogen—NH_4NO_3, KNO_3, urea, and thiourea (carbon source, glucose)—there was also very weak growth. Small concentrations of yeast autolyzate, 0.01, 0.05, 0.1, and 0.5% (by volume), had no effect on the growth of strains of the associate. All the strains were aerobic and did not form colonies submerged in the MPA (film on surface). Growth was very weak on nitrogenless agar medium.

From our study of the morphology and physiology of strains of the associate organism we were able to assign it to the species B. closteroides (Bergey, 1948).

Besides our morphological and physiological study of the associate (B. closteroides) we also made a comparative study of the physiological features of mixed (C. pasteurianum + B. closteroides) and pure cultures of C. pasteurianum.

SOME PHYSIOLOGICAL FEATURES OF MIXED AND PURE CULTURES OF CLOSTRIDIUM PASTEURIANUM

Nitrogen-Fixing Activity

A determination of the nitrogen-fixing activity of mixed cultures of C. pasteurianum showed (Table 1) that they fixed 1.5–3 times more nitrogen than pure cultures. Mixed cultures utilized all the glucose, whereas the utilization of glucose by pure cultures was 65–70% on the average. The higher nitrogen-fixing activity of the mixed cultures of C. pasteurianum and the better utilization of glucose can be attributed to the presence of the associate microorganism B. closteroides in the cultures; the joint action of C. pasteurianum and B. closteroides led to an enhancement of nitrogen-fixation and glucose consumption.

Table 1. Comparison of Nitrogen-Fixing Activity of Mixed and Pure Cultures of Clostridium pasteurianum

Strain	Amount of nitrogen fixed per 100 ml medium, mg		Amount of nitrogen fixed per g glucose fermented, mg	
	Mixed cultures	Pure cultures	Mixed cultures	Pure cultures
O146	7.78	0.56	3.89	1.01
O187	6.3	0.96	3.15	1.47
Ya306	4.75	0.47	2.37	0.69
Ya163	3.69	1.19	1.94	1.77
Ya197	5.82	1.4	2.91	2.57
K266	7.04	1.5	3.52	2.89

Behavior of Mixed and Pure Cultures of Clostridium pasteurianum Towards Different Sources of Carbon

Studies of the behavior of mixed and pure cultures towards sources of carbon nutrition showed that mixed cultures of C. pasteurianum differed considerably from pure cultures (Table 2).

In contrast to pure cultures, mixed cultures fermented glucose, maltose, lactose, dextrin, starch, glycerol, and mannitol. This effect was again attributed to the presence in the C. pasteurianum culture of the associate microorganism B. closteroides, which assisted the fermentation of these substances. As our experiments with pure cultures of the associate showed, the latter grew well not only on glucose, maltose, and sucrose, but also on lactose. According to Bergey (1948), B. closteroides also decomposes starch. Hence the associate, by decomposing carbohydrate which is difficult to ferment, assists its fermentation by the cells of C. pasteurianum.

Degeneration of Pure Cultures of Clostridium pasteurianum and the Role of the Associate, Bacillus closteroides

As we know, the medium for experiments on anaerobic nitrogen-fixation is almost always some modification of Vinogradskii's nitrogenless synthetic medium containing no growth factors. Growth on such a medium is only possible when a sufficient amount of inoculum grown on a medium containing various vitamins and other organic nutrient substances, is introduced. Inocula from cultures grown on Vinogradskii's medium or in other synthetic media are weak and in many cases give no growth. Prolonged cultivation (with frequent subculturing) of C. pasteurianum in nitrogenless mineral medium leads to the degeneration of this microorganism, with the concomitant loss of ability to sporulate and fix nitrogen (Vinogradskii, 1952; Omelyanskii, 1953; Shklyar, 1956; and others).

In our investigations with pure cultures of C. pasteurianum we also observed that after several subcultures on nitrogenless media the rate of spore formation in strains of C. pasteurianum fell considerably. We found a large number of long (several tens of microns) threadlike nonsporulating cells in the medium. Fermentation was much less vigorous.

The cultivation of such asporogenous cultures in complete complex nutrient media restored their ability to form spores. The cause of degeneration of C. pasteurianum would appear to be its inability to synthesize vitamins and growth factors. When these substances are absent from the medium the normal metabolism of C. pasteurianum is affected, and consequently the normal life cycle with spore formation is altered.

It has been reported in the literature that C. pasteurianum in mixed cultures with associate bacteria in a nitrogenless medium does not degenerate. Shklyar's investigations (1956) showed that joint cultivation of C. pasteurianum and Azotobacter in aerobic conditions led to more vigorous spore formation in C. pasteurianum.

In the view of all investigators at present, the role of associate bacteria, including B. closteroides, con-

Table 2. Growth of Mixed and Pure Cultures of Clostridium pasteurianum on Different Carbon Sources

Strain	Mixed cultures												Pure cultures									
	Glucose	Levulose	Galactose	Maltose	Lactose	Sucrose	Raffinose	Dextrin	Starch	Glycerol	Mannitol	Glucose	Levulose	Galactose	Maltose	Lactose	Sucrose	Raffinose	Dextrin	Starch	Glycerol	Mannitol
Ya36	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/+	+/+	-/-	-/-	+/+	+/+	0/0	0/0	0/0	+/+	+/+	0/0	0/0	0/0	0/0
Ya163	-/-	-/-	+/+	+/+	0/0	-/-	-/-	+/+	+/+	+/+	+/+	-/-	+/+	0/0	0/0	0/0	+/+	+/+	+/+	0/0	0/0	0/0
O146	-/-	-/-	+/+	-/-	-/-	-/-	-/-	+/+	+/+	+/+	+/+	+/+	-/-	-/-	0/0	0/0	-/-	-/-	+/+	0/0	0/0	0/0
Ya197	-/-	-/-	+/+	-/-	-/-	-/-	-/-	+/+	+/+	+/+	-/-	+/+	+/+	0/0	-/-	0/0	-/-	-/-	0/0	0/0	0/0	0/0
K217	-/-	-/-	+/+	-/-	+/+	-/-	-/-	-/-	+/+	+/+	-/-	+/+	+/+	0/0	0/0	0/0	-/-	-/-	0/0	0/0	0/0	0/0
K266	-/-	-/-	+/+	+/+	+/+	-/-	-/-	-/-	-/-	+/+	-/-	+/+	+/+	0/0	-/-	0/0	+/+	-/-	0/0	0/0	0/0	0/0
O187	+/+	+/+	+/+	-/-	+/+	+/+	+/+	+/+	+/+	-/-	+/+	-/-	-/-	-/-	-/-	0/0	+/+	-/-	-/-	0/0	0/0	0/0
Ya306	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/-	-/-	-/-	-/-	0/0	0/0	0/0	+/+	+/+	0/0	0/0	0/0	0/0

Table 3. Effect of Bacillus closteroides + Bacillus sp. on the Development of Clostridium pasteurianum Strain K266 in the Rhizosphere of Oats With and Without the Application of Fertilizer

Experimental variants	Date of analysis and phase of development			
	June 17 (time of sowing)	July 10	August 7	Sept. 30
	Experiment A (no fertilizer)			
Cl. pasteurianum, strain K266		6.0	0.36	0.46
Cl. pasteurianum, strain K266+ Bac. closteroides + Bacillus sp.	0.09 introduced with seed	10.8	0.48	0.08
	Expt. B (fertilized with sterile humus, 20% by weight of sand)			
Cl. pasteurianum, strain K266		312.0	1.22	0.72
Cl. pasteurianum, strain K266+ Bac. closteroides + Bacillus sp.	0.09 introduced with seed	2400.0	18.7	4.28

Table 4. Effect of Pure and Mixed Cultures of Clostridium pasteurianum on the Yield of Oats (Pot expt., 1959, soil culture)

Variants	Total weight of plants, g	Increase in total weight of plants due to introduction of bacteria		Included weight of grain		
		g	%	wt of grain, g	Increase in weight of grain due to introduction of bacteria g	%
Control	68.3	—	100.0	24.5		100.0
Cl. pasteurianum, strain 075	77.6	+9.3	113.5	26.4	+1.9	107.8
Cl. pasteurianum, strain 075+ Bac. closteroides	82.0	+13.7	120.0	28.6	+4.1	116.9

sists simply in the absorption of atmospheric oxygen and the creation of anaerobic conditions for C. pasteurianum. In a mixed culture with Azotobacter the latter also breaks down the metabolic products of C. pasteurianum (butyric acid, etc.) and sustains the pH of the medium at a congenial level. In addition, the associates consume the nitrogen excreted by C. pasteurianum into the surrounding medium. However, these probably do not exhaust the complex interrelationships of C. pasteurianum and its associates.

The question has now arisen of a reconsideration of the role of the associate aerobic microorganisms in the life of bacteria of the group C. pasteurianum in view of the fact that some associates, Azotobacter in particular, synthesize biotin (Krasil'nikov, 1958). Hence, it is possible that the favorable effect of Azotobacter on C. pasteurianum is due not only to the fact that it absorbs atmospheric O_2 and breaks down the metabolic products of C. pasteurianum, but mainly to the fact that it provides it with biotin. The excretion of vitamins and growth factors by Azotobacter promotes the growth of C. pasteurianum and the passage of a normal life cycle, terminating in vigorous spore formation.

Chizhik (1956) noted that aerobic microorganisms (putrefiers, yeasts, and molds) enhanced the process of butyric acid fermentation, this being accompanied by the accumulation of a large amount of granulose in the cells of Clostridium butyricum and spore formation by a greater number of cells. We know that the majority of yeasts and molds (particularly of the genera Aspergillus and Penicillium) are auxoautotrophs,

and that the vitamins synthesized by them and excreted into the surrounding medium stimulate the growth and development of C. butyricum.

Probably the role of aerobic associate bacteria consists not only in the creation of anaerobic conditions and the decomposition of metabolic products of C. pasteurianum, but mainly in the provision of the anaerobic nitrogen-fixers with vitamins and growth factors.

In our experiments with an enrichment (mixed) culture of C. pasteurianum there was no rapid degeneration. The role of the associate in this culture was apparently that of providing C. pasteurianum with vitamins and other factors. Owing to their content of various vitamins and growth factors media prepared from potato-carrot broth with yeast autolyzate and peptone restored the spore-forming ability of pure cultures.

Effect of Bacillus closteroides on the Growth of Clostridium pasteurianum in the Rhizosphere of Plants and on the Effect of Clostridium pasteurianum on Crop Yield

The inoculation of seeds of crop plants with pure cultures of C. pasteurianum (Emtsev, 1959) had a more definite favorable effect on the yield in field conditions and in soil cultures in pot experiments. At the same time the beneficial effect of C. pasteurianum was very slight in sand cultures. One of the causes of this was probably the absence of the associate microorganisms in the sand, whereas the soil provides associations of accompanying bacteria and C. pasteurianum and this enhances its activity.

In view of this we attempted to find out the effect of mixed cultures of C. pasteurianum (C. pasteurianum + +B. closteroides + Bacillus sp.*) on the yield of crops.

For the solution of this question we set up two pot experiments with oats (sand culture).

The conducted experiments showed (Table 3) that the introduction of B. closteroides + B. sp. together with the C. pasteurianum cells led to a greater multiplication of C. pasteurianum in the rhizosphere of the plants, especially when fertilizers were applied.

B. closteroides + B. sp., introduced along with C. pasteurianum into pots containing humus, stimulated the multiplication of C. pasteurianum, and thus they led to the enhancement of the beneficial effect of C. pasteurianum on the yield of the oat plants. When no fertilizer was used the aerobic associates had little effect on C. pasteurianum, and thuse we observed no appreciable increase in the yield of the oats in this variant of the experiment.

In our 1957–1959 investigations, conducted with freshly isolated strains of C. pasteurianum and B. closteroides, we confirmed by pot experiments with a soil culture of oats that C. pasteurianum, introduced with the associate B. closteroides, multiplied in the rhizosphere of plants several times better (the number of Clostridium in the mixed culture was almost ten times that of the Clostridium without the associate) than when it was introduced in pure culture. The better multiplication of Clostridium in mixed culture with B. closteroides promoted the growth and yield of the plants. As we see from Table 4, the yield of the oats when a pure culture of C. pasteurianum was introduced was 113.5% of the control, and when a mixed culture was introduced it was 120% of the control. The extra yield of grain following the introduction of the mixed culture was twice that obtained from the use of a pure Clostridium culture.

Our investigations showed that in soil conditions C. pasteurianum grows, develops and fixes atmospheric nitrogen in symbiosis with B. closteroides and, possibly, with other aerobic sporeforming microorganisms, and its high activity is manifested only when this biological environment is present.

SUMMARY

1. Bacillus closteroides is the only constant associate of Clostridium pasteurianum. B. closteroides is closely bound up with the development of C. pasteurianum and can be found in all cultures of C. pasteurianum isolated from the soil.

2. In association with C. pasteurianum, B. closteroides enhances nitrogen fixation and carbohydrate consumption, increases the number of carbohydrates consumed by C. pasteurianum, and promotes the normal development of C. pasteurianum.

3. By stimulating the vital activity of C. pasteurianum, B. closteroides enhances its beneficial effect on the yield of plants.

4. The nature of the relationships between C. pasteurianum and B. closteroides is still largely obscure. However, it may be assumed that, besides creating anaerobic conditions for C. pasteurianum, B. closteroides provides this microorganism with vitamins and growth factors. B. closteroides in turn utilizes the nitrogen fixed by C. pasteurianum.

The data given confirm Rabotnova's view (1952) that C. pasteurianum is symbiotically associated with B. closteroides.

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*The B. sp. belonged to the sporeforming bacteria of the Bacillus subtilis—Bacillus mesentericus group.

A STUDY OF THE RELATIONSHIP BETWEEN LACTOBACTERIUM BULGARICUM AND SACCHAROMYCES LACTIS

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In natural conditions microorganisms do not live isolated from one another, but develop in more or less close association with other microorganisms. The relationships within a biocoenosis vary widely from symbiosis to antagonism with various intermediate modifications. The nature of the interrelationships in microbial associations depends on the specificity of the interacting microorganisms and on the environmental conditions.

Research on the interrelationships of microorganisms is providing more and more information on particular aspects of biochemical processes due to microbes, and on the nature of the interrelationships of microbes and microassociations. This is not only broadening our knowledge of the principles governing the evolution of microorganisms, but is also opening up perspectives for practical application in various branches of industry. The published information on the relationships between lactic acid bacteria and yeasts is contradictory. Some investigators regard them as strictly symbiotic, others consider them as metabiotic, or even antagonistic. Shaposhnikov's view (1948) is interesting in this respect. He believes that the coexistence of two organisms on the same nutrient medium must lead at first to competition between them owing to their utilizing the same nutrient substances. However, in the case of lactic acid bacteria and yeasts the competition is very limited: yeasts in aerobic conditions use for their growth the lactic acid produced by the lactic acid bacteria. The lowering of the acidity of the medium has a favorable effect on the growth of the bacteria. Besides this, the yeasts are a valuable source of vitamins and amino acids, which constitute a special requirement of the lactic acid bacteria.

By means of microtome sections, Feofilova (1958) found that kefir powder is composed of lactic acid bacteria and two species of yeasts. For their development the lactic acid bacteria required certain substances contained in the yeast autolyzate and which could be adsorbed by activated carbon.

Chuzhova (1958) studied the therapeutic sour-milk drink "kurung" (a type of kumiss) and found a close biological association between lactic acid streptococci and acidophilic bacteria, on one hand, and yeasts on the other.

The majority of investigators are inclined to the view that yeasts have a beneficial effect on lactic acid bacteria. There is no doubt that this association is advantageous to both groups of microorganisms. This explains their occurrence together, an association which has developed without human interference.

Solides (1955) claims that when yeasts of the genus Torulopsis are cultured together with Streptococcus thermophilus and Lactobacterium bulgaricum in milk, the yeasts peptonize the curdled milk and reduce its acidity by their assimilation of lactic acid, as well as by their neutralization of products of proteolysis. The lactic acid bacteria, according to the data of this author, were still viable after five to eight months.

The question of the interrelationships of yeasts and lactic acid bacteria is not only of theoretical, but also of great practical interest. For instance, in the industrial production of lactic acid, as well as in the preparation of inocula (bacterial starters), it is recommended that yeasts should be added along with the homofermentative lactic acid bacteria (L. bulgaricum) in order to accelerate the process and to raise the yield of lactic acid (Prescott and Dunn, 1949).

In the present work our aim was to study the interrelationships between L. bulgaricum and Saccharomyces lactis, isolated from yoghurt. We investigated the multiplication of the bacteria and yeasts, the mean dimensions of the cells, the cultural characters, and the assimilation of compounds of carbon, nitrogen, etc.

1. DYNAMICS OF MULTIPLICATION OF LACTIC ACID BACTERIA AND YEASTS CULTURED SEPARATELY AND TOGETHER

The experiments aimed at revealing the dynamics of the multiplication of lactic acid bacteria and yeasts were carried out in the laboratory. For this purpose we used a nutrient medium equally suitable for both microorganisms, viz., hydrolyzed milk (after Bogdanov) and a girasole (Jerusalem artichoke) medium containing 4% lactose. The media were inoculated with young 24-hr-old cultures. We introduced a fixed number of cells from each culture according to a scheme worked out beforehand. The titer of the culture was determined by Koch's serial dilution technique or by the direct count method. The cultures were incubated at 42°, which was equally suitable for both microbes.

When the ratio of the cultures in the original material was 1:1 and the number of cells was small, we

found that the rates of multiplication of the bacteria and yeasts were not the same when they were grown separately and together. Thus, while the number of yeast cells in the single culture rose to $27 \cdot 10^6$ within 24 hr, their number when cultured jointly with the bacteria was $21.6 \cdot 10^6$ (Fig. 1, curves 1 and 2), i.e., there were 1.3 times more cells in the pure culture. The multiplication coefficient for yeasts in the pure culture was 862, and in the mixed culture was 656.

A rather different picture was obtained for the lactic acid bacteria. Thus, their number in the mixed culture rose to $32 \cdot 10^6$ in 24 hr, whereas in the pure culture during the same time the number rose only to $18.4 \cdot 10^6$ (Fig. 2, curves 1 and 2), i.e., was less by a factor 1.8. The multiplication coefficient of bacteria in the pure culture was 575, and in the mixed culture 1000. When the numbers of bacterial and yeast cells in the inoculum were the same, by the end of 24 hr their ratio was 1.5:1.

Thus, in the first 24 hr the lactic acid bacteria multiplied more vigorously when yeasts were present, whereas the rate of growth of the yeasts was not substantially affected. Such a relation can be considered as metabiotic, with the yeasts being the weaker partner. They create more congenial conditions for the growth of the bacteria. This conclusion was borne out by the figures for the change in actual acidity. In the mixed culture the actual acidity rose to pH 4.3, while in the pure culture of bacteria it was 4.83, and in the yeast culture 5.2.

After 48 hr of cultivation in the same conditions the differences in the rates of growth of the bacteria and yeasts were no longer so marked. More congenial conditions for the growth of the yeasts were created. The ratio of the numbers of bacteria and yeast cells became 1:1.06, i.e., almost the same as at the start of the experiment. The number of bacteria in the pure culture reached $36.1 \cdot 10^6$, and in the mixed culture it was $39.2 \cdot 10^6$. The multiplication coefficient for the bacteria in the pure culture fell to 2.1, and in the mixed culture to 1.2.

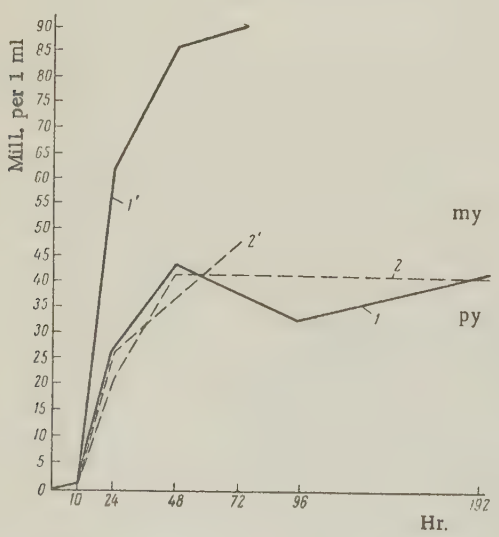

Fig. 1. Multiplication in cultures. 1, 1') Pure culture; 2, 2') mixed culture.

The number of yeast cells in the pure culture rose to $43.5 \cdot 10^6$, and in the mixed culture to $41.5 \cdot 10^6$. The multiplication coefficient fell to 1.6 for the pure culture and 1.9 for the mixed culture.

We see from the data given that both cultures multiplied most vigorously in the first 24 hr. For the yeasts the most congenial conditions for multiplication set in a little later. The presence of the yeasts can be regarded as the reason for the more rapid multiplication of the bacteria in the first 24 hr.

When we consider that by the beginning of the second day the number of cells of both microbes had become almost equal and that the conditions for multiplication of the yeasts had become better, we can say that the bacteria have become the weaker partner. The actual acidity was highest in the mixed culture (pH 3.81), while in the pure cultures it was 4.17 in the case of bacteria and 4.62 in the case of yeasts.

After 96 hr the number of bacteria in the pure culture rose to $38 \cdot 10^6$, while in the mixed culture it fell slightly ($36 \cdot 10^6$), i.e., in the pure culture there were 1.06 times more cells than in the mixed. At the same time the number of yeast cells in the pure culture fell slightly ($32.8 \cdot 10^6$), while the mixed culture maintained almost the same number of cells as at the start of the second day ($41.6 \cdot 10^6$). Thus, the number of yeast cells in the mixed culture was 1.3 times as great as in the pure culture. The ratio of the number of bacteria to the number of yeast cells in the mixed culture had now become 1:1.15. Hence, the interrelationship was still of a metabiotic nature and the bacteria were still the weaker partner.

The course of the multiplication and the changes in the numbers of bacteria and yeasts were kept under observation for 192 hr. During this time the number of bacteria fell steadily, while the number of yeast cells remained practically constant. A comparatively high actual acidity was attained in the pure culture of bacteria—pH 3.93; in the mixed culture it was 4.0. A slight reduction in the actual acidity was observed in the pure yeast culture—pH 4.71.

Hence, when L. bulgaricum and S. lactis are cultured together a variable association of a metabiotic nature is established between them. At the beginning of the experiment the yeasts are the weaker partner, and later the bacteria become the weaker partner.

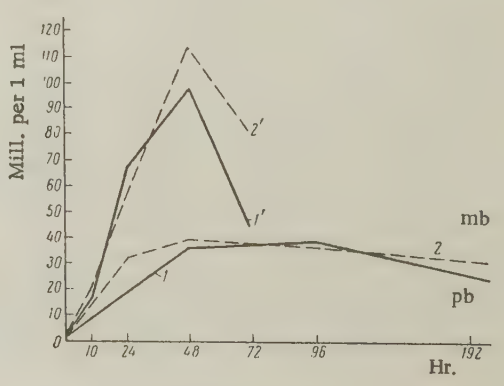

Fig. 2. Multiplication in cultures. 1, 1') Pure culture; 2, 2') mixed culture.

Special experiments were conducted to find out how the titer of the inoculum affected the interrelationships of the cultures under investigation. When the number of bacterial cells in the inoculum was 3600 times that of the yeast cells, the growth of both organisms at the beginning of the experiment (in the first 24 hr) was worse in the mixed culture than in pure cultures. The initial ratio of 3600:1 in favor of the bacteria changed to 35:1 within 10 hr and to 2.2:1 within 24 hr. A distinctive mutual inhibition was manifested, and a tendency for the numbers of bacteria and yeasts to level out was noted. The same situation prevailed till the 48th hour. After 72 hr the absolute number of yeasts was still increasing, but the number of bacteria had fallen, and more markedly in the pure culture. The ratio of the bacteria and yeasts in the mixed culture was then 1.7:1.

As we see, the introduction of an excess number of bacteria resulted in a poorer development of both microbes in the first few hours in comparison with the pure culture. The numbers of cells of each species rapidly leveled out.

In another variant of the experiment where the bacteria at the beginning were 1000 times more numerous than the yeasts, a more rapid multiplication of both cultures was observed in the mixed culture in the first 24 hr. By the end of the experiment the original ratio 1000:1 had changed to 16:1. Here we also noted a tendency for the numbers of the cells of both microbes to level out. Such a relationship can be regarded as symbiotic. In this case the introduction of bacteria and yeasts in the ratio 1000:1 was probably the most favorable.

We observed accumulations of bacteria around the yeast cells (Fig. 3).

After 42 hr cultivation the development of the yeasts in the mixed culture was strongly suppressed ($6 \cdot 10^6$), whereas in the pure culture their number rose to $20 \cdot 10^6$. The number of bacterial cells in the mixed culture by this time was twice their number in the pure culture ($75 \cdot 10^6$ and $33 \cdot 10^6$, respectively).

The initial ratio 1000:1 in the mixed culture had become 60:1 after 24 hr, and 12.5:1 after 42 hr. Hence there was an obvious tendency for the rate of multiplication of the bacteria to diminish. We also observed differences in the actual acidity of the medium. While it was 4.08 and 4.68 respectively in the pure cultures of bacteria and yeast, it rose to 3.78 in the mixed culture. The increase of acidity could probably be attributed to the higher microbial titer, and not to the higher acid-forming activity of each cell.

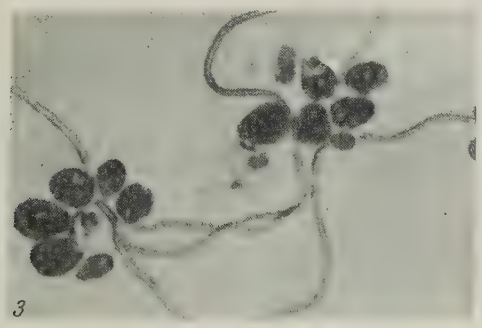

Fig. 3. Lactic acid bacteria developing around yeast cells.

When the number of yeasts in the inoculum exceeded the number of bacteria, the experiment showed that by the end of the first 24 hr the rate of multiplication of the bacteria was almost halved. Thus, while their number in the pure culture reached $32.4 \cdot 10^6$, it was $16.8 \cdot 10^6$, in the mixed, i.e., about half as many. This suppression of the multiplication of the bacteria was due to the excess number of yeasts in the inoculum, and was more pronounced the greater the excess of yeasts.

When the ratio of the initial numbers of yeasts and bacteria was 15:1, it became 2.1:1 within 24 hr, and subsequently tended towards 1:1. This relationship between bacteria and yeasts cannot be considered as metabiotic. It is quite clear that the nature of the interrelationship depends on the amount of inoculum.

2. MEAN DIMENSIONS OF CELLS OF LACTIC ACID BACTERIA AND YEASTS CULTURED SEPARATELY AND TOGETHER

In our study of the dynamics of the multiplication of microorganisms we also had to consider variations in the size of the cells. It has been found that the width (thickness) of the microbial cell is a more stable character. The variations in thickness are usually very slight and do not alter the general aspect of the cell, and hence this feature is very often used in systematics. We know also that the mean size of cells varies in relation to the age of the culture, the composition of the nutrient medium, and other conditions.

We measured batches of 40 cells in pure and mixed cultures and calculated the mean values. The mean dimensions of bacteria in the pure culture (20–22 hr) were $13.9 \times 0.96\mu$, and in the mixed culture they were a little larger ($14.3 \times 1.03\mu$). The cause of these differences in mean size must be sought in the effect of the yeasts. The cited sizes were measured in the case where the ratio of the numbers of bacteria in the pure and mixed cultures was 1:3.5, i.e., 3.5 cells in the mixed culture corresponded to one bacterial cell in the pure culture. Moreover, the ratio of bacteria and yeasts in the mixed culture was equal to 16:1. These differences in the mean dimensions (mean length) of the bacterial cells were retained up to the 40–42nd hr of cultivation. It is clear from the foregoing that each cell developing in the presence of yeasts possesses a greater surface of interaction with the nutrient medium, and this is of great importance for the osmotic feeding mechanism. The relatively larger bacterial cells in the mixed culture, however, did not appear to be freaks.

The yeast cells in the pure culture had smaller mean dimensions ($6.25 \times 4.31\mu$) than in the mixed culture ($6.53 \times 5.08\mu$) at the 20–22nd hr of cultivation. The number of cells in the mixed culture was greater than in the pure culture. By the 40–42nd hr of cultivation the growth of the yeast cells in the presence of the

bacteria was arrested. For instance, while the mean dimensions of the yeast cells in the pure culture were $7.08 \times 5.85\mu$, in the mixed culture they were $6.32 \times 5.67\mu$. This shows that cultivation of lactic acid bacteria together with the yeasts could lead not only to changes in the dynamics of multiplication, but also to a change in the mean dimensions of the cells.

3. OBSERVATIONS ON THE CULTURAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERS OF LACTIC ACID BACTERIA AND YEASTS IN PURE AND MIXED CULTURES

In Fedorov's opinion (1954) the onset of dissociation in bacteria is promoted not only by moisture, temperature, the nutrient substances, and the reaction of the medium, but also by products of the vital activity of the microbes. This is very easily observed in liquid nutrient media. The suggestion of some investigators that dissociation may be due to contamination of the cultures is not always justified, since pure cultures produced from a single cell are usually employed.

In our experiments we investigated the changes of form of colonies of L. bulgaricum and S. lactis cultured separately and together. The subcultures were made after 22–36 hr since, as we know, the action of any factor on the microbial cell is very pronounced if it coincides with the period of growth and intense assimilation of nutrient substances. The cultural characters of bacteria and yeasts were studied on agar containing hydrolyzed milk (after Bogdanov) and girasole agar medium with pH 6.0–6.5.

We found differences in the form and dimensions of the lactic acid bacteria colonies after prolonged (seven months) cultivation with yeasts. While bacteria in pure culture formed the rough colonies (R type) characteristic of this species, their colonies in a mixed culture had a more rounded form and showed a tendency toward the S type (Figs. 4 and 5). In Krasil'nikov's opinion, the transformation of R forms into S variants is possible.

We also found differences in the size of the colonies. While the colonies of lactic acid bacteria in pure culture had a mean diameter 257μ (from 215 to 295μ), their mean diameter in mixed culture with yeasts was 451μ (from 360 to 520μ). The doubling of the diameter of the colonies when yeasts were present is a definite inidcation that yeasts create more congenial conditions for the bacteria. Bogdanov (1954) also observed an increase in the size of colonies of a lactic acid streptococcus when it was cultured together with yeasts of the genus Mycoderma.

We should note that these differences in the form of the colonies were retained only through eight to ten subcultures of the pure culture, and then the colonies returned to the original form typical of the species. In the yeast colonies we noted no differences in this respect.

Many investigators believe that the obtention of far-reaching changes and the development of new useful strains and species can be realized more easily and more rapidly with microorganisms than with higher plants and animals. We could cite numerous examples of the obtention of new forms of microorganisms. In view of this it was of interest to find out if the observed morphological and cultural changes in our cultures were accompanied by changes of a physiological or biochemical nature.

We see from the table that when the lactic acid bacteria was cultured in milk (variant 1) they fermented glucose, galactose and lactose. After 15 days' subculturing on girasole liquid medium they began to ferment, though inconsistently, sucrose and maltose. This clearly shows that the nutrient medium can affect the fermentative ability of the bacteria.

After seven months' joint cultivation with yeasts, maltose fermentation by the bacteria was a stable feature, but sucrose fermentation was still variable (variant 3 in table). We note that they acquired the ability to ferment levulose, though this was variable. These changes of fermentative ability were accompanied by slight changes in colony form, a feature which we mentioned earlier.

After 80 days' cultivation of the lactic acid bacterium when it had been again isolated from the mixed culture with yeasts (variant 4), it was found to have lost the ability to ferment sucrose and levulose and was similar in physiological properties to the pure original culture (variant 5), which served as a control throughout the experiment. The results obtained show that the properties acquired in mixed culture with yeasts were of an adaptive nature, and hence when the bacteria were isolated from the mixed culture, their fermentative properties gradually reverted to those of the original culture. Probably the time which we chose for join

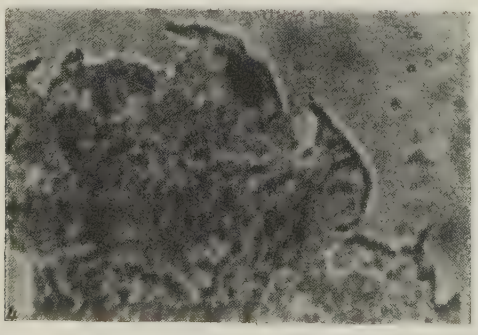

Fig. 4. Typical R colony of Lactobacterium bulgaricum (pure culture).

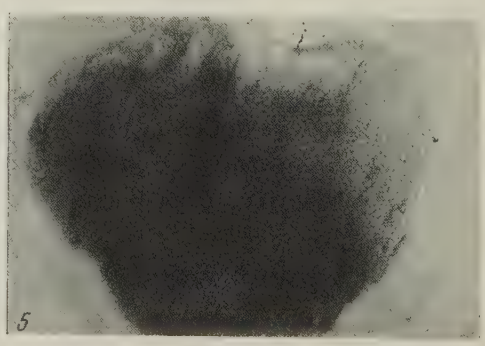

Fig. 5. Lactobacterium bulgaricum colony after seven months' cultivation with Saccharomyces lactis.

Fermentation of Sugars by Lactic Acid Bacteria in Pure Culture and in Mixed Culture with Yeasts

Variant	Culture	Duration of cultivation, days	Sugar													
			glucose	galactose	sucrose	maltose	levulose	lactose	xylose	arabinose	inositol	glycogen	inulin	glycerol	mannitol	dextrin
1	Pure	—	±	±	—	—	—	—	—	—	—	—	—	—	—	—
2	"	15	+	+	±	+	—	+	—	—	—	—	—	—	—	—
3	Mixed	210	+	+	—	+	+	+	—	—	—	—	—	—	—	—
4	Pure	80	+	+	—	+	—	+	—	—	—	—	—	—	—	—
5	"	—	+	+	—	+	—	+	—	—	—	—	—	—	—	—

cultivation (210 days) was not long enough to produce stable heritable changes in the bacteria. The important point here is that the continued presence of yeasts had some effect on the enzyme systems of the lactic acid bacterium, and hence on its metabolism, i.e., in joint cultivation one microorganism can affect the other.

We must mention that Prescott and Dunn (1949) also reported that the fermentation of levulose, maltose, and sucrose by this lactic acid bacterium was variable and depended on the strain and culture conditions.

When L. bulgaricum and S. lactis were cultured together we found no differences in the assimilation of nitrogen compounds in comparison with pure cultures. This means that the proteolytic enzymes of these bacteria are more stable.

Prolonged (seven months) joint cultivation of the two cultures led to a loss of ability of the yeasts to ferment inulin, but in return for this they acquired the ability to ferment maltose. After 80 days' growth in pure culture the yeasts again lost the ability to ferment maltose. As a whole, the enzyme system of yeasts in this case appeared more stable than that of the bacteria.

The isoelectric point of the lactic acid bacteria in pure culture in the first 24–36 hr in most cases was lower than in mixed culture (2.3–2.8 against 2.8–3.0). After 72 hr of cultivation the isoelectric point of the bacteria in pure culture had risen, but in the mixed it remained at its former level. In yeasts we found no such differences in the isoelectric point—usually its value lay between 3.0 and 3.8. These data confirm Ružička's opinion that as microbial cells age the isoelectric point shifts towards the neutral point.

In almost all our experiments we observed an increase in the viability of lactic acid bacteria when yeasts were present. In individual cases the culture retained its viability for 18 months at room temperature.

From experiments carried out to reveal the causes of the favorable effect of yeasts on lactic acid bacteria, we managed to establish that yeasts were a source of the following amino acids: cystine, lysine, arginine, histidine, aspartic acid, glutamic acid, threonine, alanine, proline, tryptophan, methionine, valine, phenylalanine, leucine, as well as vitamins, and in some cases yeasts emerged as consumers of acids. Joint cultivation of L. bulgaricum and S. lactis led to an enhancement of the antimicrobial properties of the lactic acid bacterium towards certain Gram-positive and Gram-negative bacteria.

SUMMARY

1. A pure culture of Lactobacterium bulgaricum does not develop so well on hydrolyzed milk or girasole medium as a culture mixed with Saccharomyces lactis. A variable association, usually of a metabiotic character, is set up between the microorganisms, the yeasts being the weaker partner at the start of cultivation, and later on the bacteria.

2. The quantity of bacteria and yeasts in the inoculum affects their interrelationships.

3. Prolonged joint cultivation of the two cultures can lead to morphological and physiological changes, and also to biochemical changes.

4. The favorable effect of yeasts on the development of lactic acid bacteria can be attributed to the fact that yeasts are a valuable source of amino acids and vitamins.

5. When yeasts are present the lactic acid bacteria can retain their viability at room temperature for 18 months in some cases.

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THE SIGNIFICANCE OF INVOLUTION FORMS OF BACTERIA IN THE EVALUATION OF THEIR SUSCEPTIBILITY TO ANTIBIOTICS

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The study of polymorphism in bacteria has been the subject of numerous investigations, the early ones being the classic experiments of Gamaleya (1894) on the action of lithium salts on bacteria.

Morphological changes in bacteria exposed to antibiotics can be classed as manifestations of bacterial polymorphism. These were observed by Emmerich and Saida (1900) in a study of the effect of pyocyanase on the anthrax bacillus.

Welch et al. (1946) observed unusually large forms in cultures of these microbes subjected to the action of streptomycin.

Using time lapse cinemicrography Troitskii and Pershina (1949) analyzed the formation of involution forms in bacteria exposed to penicillin.

From a close examination of these morphological changes we can conclude that processes of growth and division of bacterial cells depend on various factors and, in particular, may be modified by the action of antibiotics.

Gardner (1940, 1945) believes that the formation of involution bacterial cells in the presence of penicillin is due to the continuing growth of the cells when their division is suppressed.

Heteromorphic forms of bacteria also appear when bacteria are acquiring resistance to antibiotics, as was shown by Moroz (1959) in the case of a grizemin-resistant* variant of the colon bacillus and by Rubinshtein (1958) in the case of a streptomycin-resistant vaccine strain of the plague bacillus.

Most of the published information merely affirms that involution forms of bacteria can be obtained, but very little attention has been given to the practical use of these important cytological facts.

In view of this we set ourselves the task of studying the production of involution forms of bacteria in order to use this feature for the elaboration of a new and rapid method of determining the susceptibility of bacteria to antibiotics by means of phase-contrast microscopy.

We used the coli-typhoid group of bacteria and determined their susceptibility towards the tetracycline antibiotics tetracycline, chlortetracycline (biomycin), and oxytetracycline (terramycin).

As the basis of our tests on the investigated preparations with the phase-contrast microscope we made use of the known facts that normal bacterial microcolonies are found in microchambers after four to five hours' incubation at 37°, and that giant involution forms are produced under the action of antibiotics. This agreed with the results which we obtained by means of time lapse cinemicrography, where we found that bacteria of the coli-typhoid group gave rise to involution forms in a short period time, not exceeding four to five hours, when they were exposed to the action of tetracycline antibiotics.

In our investigations the microchambers were made of agar containing a fixed concentration of the antibiotic, and were inoculated with a suspension containing 2 billion cells of the bacteria under test.

The number of microchambers used in the experiment corresponded to the usual number of test tubes in the serial dilution method, including the control microchamber (no antibiotic).

The microchambers with the test bacteria were installed in an incubator at 37° for 4–5 hours. On the elapse of this time the preparations were examined under the phase-contrast microscope.

The results obtained were recorded by photomicrography. The susceptibility of the control was determined by serial dilutions of the same antibiotics with the above-mentioned bacteria.

The control determinations were made after 18–20 hours' incubation.

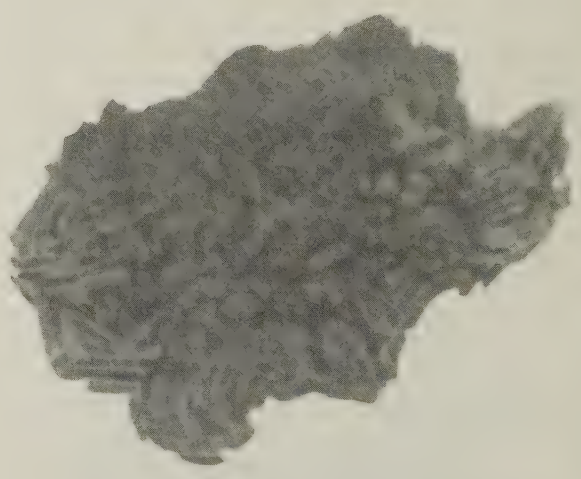

Fig. 1. Normal microcolony of paratyphoid bacillus B. Photomicrograph. Mag. 600 ×.

*Transliteration of Russian. Possibly should be grisein—Publisher.

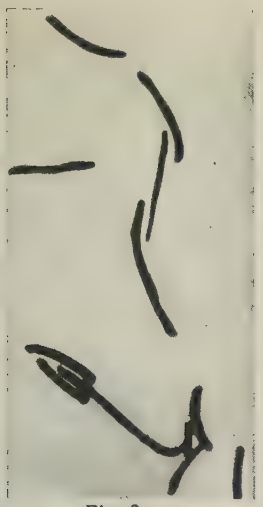

Fig. 2.

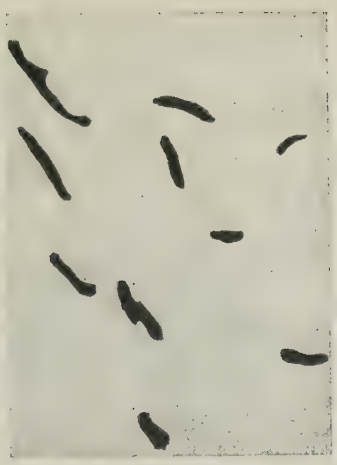

Fig. 3.

Fig. 2. Formation of involution forms of paratyphoid bacillus B under the action of biomycin. Photomicrograph. Mag. 600×.
Fig. 3. Paratyphoid bacillus B exposed to bacteriostatic dose of biomycin. Mag. 600×.

We found that normal microcolonies were formed in those microchambers where the agar contained no antibiotic at all (control microchambers), or where the antibiotic was present in subbacteriostatic doses (Fig. 1).

Besides this, in the microchambers where the dose of antibiotic arrested the division of the bacterial cells, but where the mass of the bacterial cells continued to increase, we noted the production of giant involution forms, which appeared as a result of the action of tetracycline antibiotics on the coli-typhoid bacteria (Fig. 2).

By comparing the results obtained in tests of the susceptibility of bacteria to antibiotics in the microchambers with the results of the serial dilution method we concluded that the bacterial polymorphism due to the action of antibiotics was most pronounced in those microchambers which corresponded to the serial dilution tubes containing the minimum dose of antibiotic required to suppress the growth of the bacteria (table).

Table. Susceptibility of Tested Bacteria to Oxytetracycline (γ/ml)

Strains	Susceptibility determined by	
	Phase-contrast microscope	Serial dilution method
Bact. dys. Flexner № 550	0.3	0.3
Bact. typhi abd. № 2406	0.3	0.15
Bact. Breslau № 2311	0.7	1.5
Bact. Paratyphi № 1282	0.7	1.5

From this table it is clear that there is a direct relationship between the concentration of antibiotic in the agar of the microchamber, and the appearance of giant involution forms.

Besides this, in chambers with a high concentration of antibiotic the bacterial cells were dispersed and did not form microcolonies (Fig. 3).

Thus, among the microchambers containing the bacteria under investigation the one in which the polymorphism was most pronounced was a boundary case, and the concentration of antibiotic in it, as we see from the table, corresponds to the minimum concentration of antibiotic required for inhibiting the growth of the bacteria, i.e., to the susceptibility of the tested bacteria to the antibiotics.

The principle of our method of testing the susceptibility of bacteria to antibiotics by means of the phase-contrast microscope is based on a consideration of the appearance of involution forms of bacterial cells from a new angle and its correlation with the measured susceptibility to antibiotics.

SUMMARY

1. A comparative study was made of the susceptibility of bacteria of the coli-typhoid group to tetracycline antibiotics by the serial dilution method and by phase-contrast microscopy. The results obtained by the two methods were close.

2. A new principle is proposed for rapid testing of the susceptibility of bacteria to antibiotics by means of phase-contrast microscopy. It is based on a determination of the minimum concentration of antibiotic which leads to the appearance of involution forms of bacteria within four to five hours.

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PHYSIOLOGICALLY ACTIVE SUBSTANCES FROM THE TREE FUNGUS POLYPORUS BETULINUS (BULL.) KARST

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Among the tree fungi which we are studying in our laboratory in the quest for physiologically active substances, the birch tree fungus Polyporus betulinus is an object worthy of attention. It contains the polyporenic acids A and C, which possess antibiotic properties against several bacteria (Locquin, 1948; Marcus, 1952). P. betulinus belongs to the wood-destroying tree fungi, which cause a yellowish brown or reddish brown rot in trees. It grows on dead and, very rarely, on living birches (Bondartsev, 1953).

The polyporenic acids A and C belong to the triterpene group—natural compounds of vegetable origin. Acid A ($C_{31}H_{50}O_4$) contains one carboxyl group, two hydroxyl groups and two unsaturated bonds. As distinct from acid A polyporenic acid C ($C_{31}H_{46}O_4$) contains one hydroxyl and one carbonyl group.

The polyporenic acid A which we isolated was active against Staphylococcus aureus 209 in a dilution of 1:300,000, and against Escherichia coli in a dilution of 1:2,000,000. Polyporenic acid C was slightly active against S. aureus and E. coli (1:5000 and 1:10,000).

The aim of the present work was to discover the effect of the polyporenic acids A and C and a third component, isolated from P. betulinus, on fermentation by yeast and the growth of mold mycelium.

METHODS

Isolation of polyporenic acids

Ground air-dried fungus was extracted with petroleum ether and then with ethanol. Some of the polyporenic acid A passed into the petroleum-ether fraction, but the bulk of it was contained in the alcohol fraction owing to its better solubility in alcohol than in ether. The acid was separated from the alcohol solution by the somewhat modified method described by Curtis, Heilbron, et al. (1953). The alcoholic residue obtained after removal of the alcohol was hydrolyzed for five hours with alcoholic potassium hydroxide, the hydrolyzate was diluted with water, and the neutral substances (not saponifiable) were separated from it by shaking with ether. The hydrolyzate was then acidified with HCl and the acids precipitated were transferred into ether. The ether residue was treated with glacial acetic acid for the removal of gummy substances, and then with activated carbon in alcohol solution. The amorphous residue obtained after removal of the alcohol was subjected to repeated crystallization from aqueous methanol or aqueous acetone. At first there was a precipitate of some acid less soluble in alcohol and acetone than acid A. A positive Liebermann-Burchard reaction and the similarity of the melting point (285–288°) justified the assumption that this acid was "eburicoic" or tumulosicoic acid, found in many tree fungi. After removal of this acid from the mother liquor we obtained polyporenic acid A with a melting point 195–198°. Acid A possessed antibiotic activity against several Gram-positive bacteria. The "eburicoic" acid was inactive.

For obtaining polyporenic acid C the remains of the fungus after alcoholic extraction were extracted with acetone. From the acetone extract the acid was isolated by one of the methods described by Bowers, et al. (1953). First we obtained the methyl ester of the acid, which was then hydrolyzed with methanolic potassium hydroxide and the free acid produced was purified by passage through a column containing alumina. After this treatment the acid obtained in crystalline form had a melting point consistent with the published data (270°).

The polyporenic acids were slightly soluble in cold water, and easily soluble in acetone, ethanol, and methanol. Solutions of the acid in 70% ethanol were used for the tests.

The vigor of the fermentation was assayed from the amount of CO_2 evolved in the fermentation liquid within a fixed period time. The experiments were conducted in flasks with a Meissl valve and a Bunsen collar (Ivanov, 1942). We used 10% sucrose solutions containing 0.5 g of baker's yeast. The volume of the fermentation liquid was brought to 50 ml. To allow for the effect of alcohol introduced with the preparation, we added an equal amount of alcohol to the control sample in cases where the alcohol content of the sample was more than 1%.

The weighings were made every 3–4 hr over 24 hr.

EFFECT OF TRITERPENIC ACIDS ON FERMENTATION BY YEAST

We tested various concentrations of the acids. We found that they showed a stimulating effect in a fairly wide range of concentrations—from 0.0001 to 0.01%.

Figure 1 shows the course of fermentation by yeast with polyporenic acid A added, Fig. 2 with polyporenic acid C added, and Fig. 3 with "eburicoic" acid added.

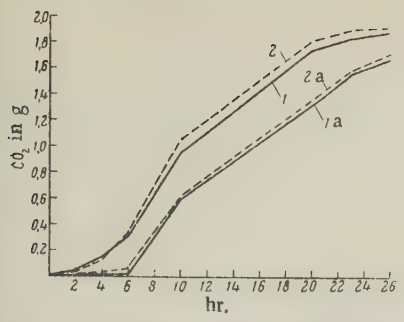

Fig. 1. Dynamics of sucrose fermentation with polyporenic acid A present. 1) 1.0 mg %; 1a) control; 2) 2 mg %; 2a) control.

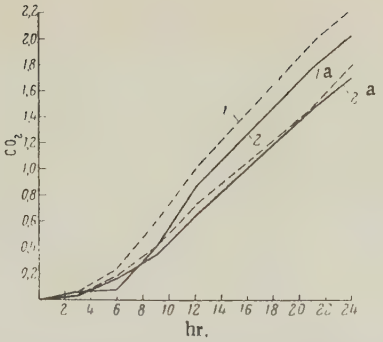

Fig. 2. Dynamics of sucrose fermentation with polyporenic acid C present. 1) 10 mg %; 1a) control; 2) 4 mg %; 2a) control.

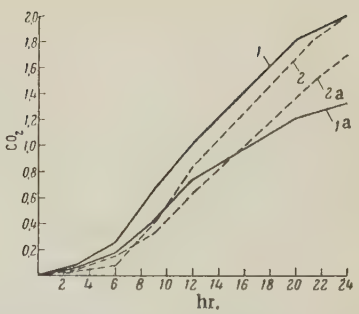

Fig. 3. Dynamics of sucrose fermentation with "eburicoic" acid present. 1) 8 mg %; 1a) control; 2) 4 mg %; 2a) control.

Effect of Triterpenic Acids on the Growth of Molds.

Organism	Control	Polyporenic acid A		Polyporenic acid C		"Eburicoic" acid	
	dry wt of mycelium	dry wt of mycelium	increment in %	dry wt of mycelium	increment in %	dry wt of mycelium	increment in %
1. Asp. niger	0.142	0.178	25	0.179	26	0.174	22.5
2. " "	0.262	0.322	22.9	0.317	21	0.314	20
3. Pen. chrysogenum	0.124	0.152	22.5	—	—	0.156	25.8

As the given results reveal, the triterpenic acids began to have some effect in the first few hours of fermentation. In the case of acid A the fermentation curves in the experimental and control samples had a parallel course, with the control lagging by 12% as regards CO_2 production. Polyporenic acid C stimulated fermentation by 18.25–22.3%, "eburicoic" acid by 17.6 to 46.6%. The stimulating action of the two last acids, insignificant at the start of fermentation, gradually increased during the whole observed period of fermentation (24 hr).

EFFECT OF TRITERPENIC ACIDS ON THE GROWTH OF MOLDS

As objects for the experiment we used Aspergillus niger and Penicillium chrysogenum of the "New Hybrid" strain.

The fungi were grown in surface cultures in 100 ml flasks, which contained 50 ml of nutrient (Czapek for the Aspergillus, and the synthetic medium employed in industry for Penicillium). Dilute solutions of the acids were added to the experimental flasks. We found that the optimum concentrations for stimulating the growth of the fungal mycelium were 0.001 to 0.005% of the nutrient. A higher acid content (from 0.05 to 0.01%), though not reducing the weight of the mycelium, retarded its growth by approximately 24 hours in comparison with the control. At the stage when spore fermentation began the mycelium was removed, washed on a filter and dried.

The table gives the data of three experiments (average values from two replicates).

As the table shows, triterpenic acids increased the yield of dry mycelial mass by 2 to 26%.

SUMMARY

1. Polyporenic acids A and C and "eburicoic" acid, isolated from a tree fungus and belonging to the group of triterpenic compounds, have a stimulating effect on fermentation by yeast and growth of mold mycelium.

2. These acids are effective in very small concentrations, from 0.0001–0.001%.

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SOME DATA ON THE OCCURRENCE OF AZOTOBACTER IN THE SOILS OF THE GORNO-ALTAI AUTONOMOUS OBLAST

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The soil cover of the Gorno-Altai Autonomous Oblast is fairly diverse. Here we find podzolic, chernozem, chernozem-like, loamy, chestnut, solonchak-like, half-bog, coarsely skeletal and other soils (Panfilov, 1956; Ul'yashchenko, 1937).

Many of the above soils, owing to their rich natural properties, are certainly of great agricultural value. The physicochemical properties of these soils have been studied to some extent but there is no information on the microbes, and on Azotobacter in particular, in the soils of the Gorno-Altai Autonomous Oblast, with the exception of some data in Blinkov's works (1955).

We have been conducting investigations to find out to what extent Azotobacter occurs in the soils of the crop fields on collective and state farms in certain districts of this region. The occurrence of Azotobacter was investigated over a period of two years (1957–1958).

In most cases the soil samples were subjected to microbiological analysis immediately after they were taken in the field.

In our experiments to discover Azotobacter we used the method of soil plates, prepared according to Vinogradskii. The soil plates were prepared without drainage. Some aeration was provided by small depressions made on the surface of the plates with the end of a glass spatula. The colonies were counted after 6–10 days' incubation at 27–29°.

At the same time we investigated the acidity of the soils of certain districts.

We studied the soils in the crop fields of the Kirov Collection Farm in Maima District. These soils were chernozem-like, meadow, alluvial sandy clay and mountain-chernozem. Fertilizers had been applied under some of the crops. For instance, in 1957 0.5 centner of ammonium sulfate per ha and compost had been applied on the potato field. In 1958 11 tons of compost per ha and 0.5 centner of ammonium nitrate had been applied to the maize field.

The results of the investigations are shown in Table 1.

The figures given in Table 1 show that Azotobacter is most abundant in chernozem-like and mountain chernozem soils.

We should note that in May, Azotobacter could not be found in any sample of the investigated soils, this being obviously due to the low temperature of the soil at the time of examination.

We also studied the occurrence of Azotobacter in soils of the Choiskii Beef State Farm in Maima District. The soils of this district were predominantly podzolic and loamy. Half-bog soils were also present. The soil on the crop fields of the State Farm was loamy. The results are given in Table 2.

The data of Table 2 show that Azotobacter occurs in very small numbers or is completely absent in the

Table 1. Azotobacter in the Soils of the Kirov Collection Farm in Maima District

Crop	Soil	Soil pH	Date of sampling	Number of colonies per plate
Oats and clover	Mountain chernozem	5.6	June 7, 1957	80
Clover	" "	5.6	June 7, 1957	6
Oats	" "	5.6	May 23, 1957	0
Clover, 2nd year	" "	5.6	May 23, 1957	0
Clover, 2nd year	" "	5.6	July 12, 1958	9
Clover, 1st year	" "	5.6	May 23, 1958	0
Clover, 1st year	" "	5.6	July 12, 1958	6
Potatoes	Chernozem-like	5.8	June 7, 1957	9
Potatoes	" "	5.8	July 12, 1958	117
Maize	Alluvial sandy clay	5.4	June 7, 1957	3
Maize	" " "	5.4	May 23, 1958	0
Maize	" " "	5.4	July 12, 1958	15
Winter rye	" " "	5.4	June 7, 1957	3

Table 2. Azotobacter in Loamy Soils of the Choiskii Beef State Farm in Maima District (1958)

Crop	Soil pH	Date of sampling	Number of colonies per plate
Oats after long fallow	5.6	June 10	24
Oats after long fallow	5.6	Sept. 4	12
Oats after long fallow	5.4	Oct. 10	0
Maize	4.2	June 10	0
Maize	4.2	Sept. 4	0
Maize	4.2	Oct. 10	0
Oats after potatoes	4.2	June 10	0
Oats after potatoes	4.2	Sept. 4	0
Oats after potatoes	4.2	Oct. 10	0
Oats after clover	5.8	June 10	27
Oats after clover	5.8	Sept. 4	3
Oats after clover	5.8	Oct. 10	2

Table 3. Azotobacter in the Soils of the Variety Test Plot of Maima District (1957)

Fields	Fertilizers	Time of sampling	Number of colonies per plate
"Diamant" wheat	Compost 10 ton/ha, superphosphate 3 centner/ha, potassium chloride 1.5 centner/ha	June 28	75
"Pobeda" oats	Superphosphate 3 centner/ha, potassium chloride 1.5 centner/ha	June 28	332
Maize and soya	Superphosphate 3 centner/ha, potassium chloride 1.5 centner/ha	June 28	57
Clover	Ash 3 centner/ha, superphosphate 2 centner/ha	June 28	60
Oats and vetch	Superphosphate 3 centner/ha	June 28	198

Table 4. Azotobacter in Chernozem Soils of the "Hero of Labor" Collective Farm in Elikmonar District (1958)

Crop	Time of sampling	Number of colonies per plate
Potatoes	June 6	9
"	Aug. 29	0
Wheat	June 6	7
"	Aug. 29	3
Oats	June 6	6
"	Aug. 29	0
Clover	June 6	306
"	Aug. 29	267
Maize	June 6	21
"	Aug. 29	9

Table 5. Azotobacter in the Soils of Shebalino Deer State Farm in Shelbalino District

Crop	Soil	Soil pH	Time of sampling	Number of colonies per plate
Potatoes	Chernozem-like	5.6	June 21, 1958	19
"	"	5.6	Oct. 1, 1958	45
Sunflowers	Exhausted chernozem	5.6	June 21, 1958	18
"	" "	5.6	Oct. 1, 1958	3
Oats and peas	Chernozem-like	5.8	June 21, 1958	6
" " "	"	5.8	Oct. 1, 1958	0

Table 6. Azotobacter in the Soils of the Agricultural Experimental Station (Date of sampling, July 12, 1957)

Field	Number of colonies per plate
Fallow	18
Potatoes	9
Perennial grasses	126
Melons	3
Perennial grasses	24
Maize	138
Oats and perennial grasses	33

soils of fields under various crops on the Choiskii Beef State Farm.

Moreover, these investigations on the occurrence of Azotobacter show a reduction in the numbers of Azotobacter or its complete absence in October, this being evidently due to the lowered temperature of the soils.

The absence of Azotobacter in soils under maize and under oats following potatoes was probably due to the high acidity of these soils.

In Maima district, besides the soils from the fields of the state and collective farm, we also examined samples of soil from the fields of the variety test plot. The soils of this plot were predominantly chernozem-like and chernozem.

The results of these investigations are given in Table 3.

The data in Table 3 indicate that Azotobacter was abundant in soils of the variety test plot, this being due to the systematic application of fertilizer and the employment of proper agricultural techniques.

Table 4 gives data on the presence of Azotobacter in the soils of the "Hero of Labor" Collective Farm in Elikmonar District.

The soil on the fields of this collective farm was chernozem.

The figures in Table 4 show that the greatest numbers of Azotobacter were found in the field under clover.

Moreover, from the data in the Table we see that the numbers of Azotobacter in the soils of the fields under all crops fell towards the end of August.

We also studied the occurrence of Azotobacter in soils of the fields of the Shebalino Deer State Farm in Shebalino District.

The results of this investigation are shown in Table 5.

The results of this investigation, given in Table 5, revealed the presence of Azotobacter in the soils of the fields of Shebalino Deer State Farm. It was most abundant in chernozem-like fertilized soil.

The table reveals that the numbers of Azotobacter in the soils under all crops also fell towards October.

The results of an investigation of soil samples taken from the crop fields of the Agricultural Experimental Station (Kyzyl-Ozek) are given in Table 6.

The soils of the crop fields were meadow-chernozems, which had been systematically fertilized with manure.

As Table 6 shows, the numbers of Azotobacter in the soils under various crops were not the same. The greatest numbers of Azotobacter were found in soils under perennial grasses and maize.

SUMMARY

1. A study was made of the occurrence of Azotobacter in the soils of Maima, Shebalino, and Elikmonar Districts of the Gorno-Altai Autonomous Oblast. It was found that this organism was most abundant in chernozem-like and mountain chernozem soils.

In loamy soils it occurred in very small numbers or was absent altogether. The absence of Azotobacter in these soils was due to their high acidity.

2. The numbers of Azotobacter in the investigated soils, as observations showed, fell towards autumn.

3. Systematic application of fertilizers and proper agricultural techniques promote the development of Azotobacter.

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OCCURRENCE OF AZOTOBACTER IN CRIMEAN SOILS

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The soils and vegetation of the Crimean Peninsula are extremely diverse. Five natural regions are represented in the Crimea, but four-fifths of the whole territory belongs to the steppe region and only one fifth to the forest-steppe, mountain-forest, mountain and south coast regions.

The present soils of the peninsula (Klepinin, 1935; Ivanov, 1958) consist of southern chernozems, meadow-chernozems and chestnut soils, solonetz and solonchaks, forest-steppe soils of the foothills, mountain-meadow soils on the mountain pastures, and brown and reddish brown soils of the south coast.

We do not know of any systematic studies dealing with Azotobacter in the Crimean soils.

In Sushkina's monograph and review (1949, 1952), and in Mishustin's works (1953, 1954), which gave extensive data on samples of various soils from many regions of the Soviet Union, no reference was made to their having investigated Crimean soils for the presence of Azotobacter. The results obtained by other investigators who have studied this question are very contradictory.

Kostychev et al. (1926), in a study of the soils of the south coast of the Crimea (Nikita Botanical Gardens), found considerable numbers of Azotobacter in them, and they ascribed to Azotobacter an important role in providing the tobacco plantations with nitrogen. In contrast to these authors Krasil'nikov (1954) believes that Azotobacter is rare or absent altogether in the soils of the south coast of the Crimea. Only very small numbers of Azotobacter were found by Simakov (1932) in the mountain-meadow soils of the Crimea, and by Avdeeva (1954) in the soil of a deep-plowed plot for a vineyard in the steppe region of the Crimea, and then only in spring and autumn.

In Sushkina's opinion (1949), which is based on published information, Azotobacter is abundant in cultivated Crimean soils, and is totally absent from virgin soils.

Below we give the results of our study of the occurrence of Azotobacter in soils of the steppe, foothill, and part of the mountain zone of the Crimea.

METHODS

We investigated the following types of soil: solonchaks, solonetz, chestnut and dark chestnut soils, southern chernozems, carbonate and leached, meadow-chernozem and mountain-meadow soils.*

We examined soils from virgin and fallow fields, as well as from those under perennial grasses, grain, and intertilled crops, cotton, and from orchards, vineyards and market gardens.

We studied both irrigated and unirrigated areas.

The soil samples were taken from different depths, which are given for each sample in the tables. We investigated soils of different sections and average samples of several sections. Altogether we collected 1400 soil samples, from which 260 average samples were made up and analyzed.

The soil was seeded onto nitrogenless media—Vinogradskii's silica gel and Ashby agar (Fedorov's modification). When there was no growth of Azotobacter on these media we prepared soil plates, which enabled us to discover Azotobacter when the numbers in the soil were very small. The count of the Azotobacter was based on a measured weight of soil, and the results converted to 1 g of dry soil.

RESULTS OF INVESTIGATIONS

The data on the distribution of Azotobacter in the chestnut-soil zone of the Sivash depression (Dzhankoi, Krasnoperekopsk, and Razdol'noe Districts) are given in Table 1. As the figures in Table 1 reveal, Azotobacter was absent from secondarily solonchakized solonetz and from puffy solonchak. In chestnut solonetz and in dark chestnut soil, Azotobacter was found in considerable numbers. In meadow solonetz soil the greatest numbers of Azotobacter (4400 per 1 g) were found at depth 20–30 cm (horizon B), which agrees with the results of Sabinin and Genkel' (1927), and Genkel' and Danini (1935).

In cultivated soils at various stages of transition towards solonetz, Azotobacter was found in almost all the samples, although usually in very small numbers. In most cases, irrigation promoted an increase in the numbers of Azotobacter in the soil.

Thus, we found Azotobacter both in cultivated, as well as in virgin chestnut soils, which does not bear out Sushkina's data, indicating that Azotobacter is absent in virgin soils of the Crimea. It is possible that the data given by Sushkina referred to other types of Crimean soils, or that the samples investigated were collected in the period when Azotobacter was in

*Some soil samples were kindly placed at our disposal by Prof. V. N. Ivanov, Head of the Soil Science Department.

Table 1. Occurrence of Azotobacter in Chestnut Soils

Sampling site	Soil	Crop	Time of sampling	Depth of sampling, cm	Numbers of Azotobacter	Notes
Steep slope towards Sivash near Chongar bridge	Secondarily soloncha-kized solonetz	Uncultivated steppe	June	0–25 25–50	0 0	Samples taken after period of rain
Lowland near "Solenoe ozero" station	Chestnut puffy solonchak	Uncultivated steppe	June	5–20	0	Samples taken after period of rain
Steppe near "Solenoe ozero" station	Solonchakized very solonetz-like chestnut soil	Uncultivated steppe	June	0–10 10–25 25–40	5300 410 37	Samples taken after period of rain
Meadow 125 km north of Simferopol	Meadow solonetz-like soil	Uncultivated steppe	June	5–15 20–30	910 4400	Samples taken after period of rain
Steppe near auto highway 6 km south of Dzhankoi	Slightly washed-out dark chestnut	Uncultivated steppe	June	5–15 20–25 35–40	460 23 0	Samples taken after period of rain
Razdol'noe District, "Red October" Collective Farm	Chestnut, weakly solonetz-like	Second-year crop of alfalfa	October	0–40 60–70	0 12	Unirrigated area
Razdol'noe District, "Red October" Collective Farm	Chestnut, weakly solonetz-like	Deep-plowed to depth 70 cm	October	10–20 40–70 80–145	0 10 0	Unirrigated area
Razdol'noe District, "Red October" Collective Farm	Chestnut, weakly solonetz-like	Field plowed after cotton	October	0–20 20–45	320 48	Irrigated area
Razdol'noe District, "Red Partisan" Collective Farm	Chestnut, moderately solonetz-like	Winter wheat following fallow	October	0–15 20–30	0 15	Unirrigated area
Krasnoperekopsk District, "Red" Collective Farm	Chestnut, solonetz-like	Market garden	October	0–20 20–30	100 0	Unirrigated area
Krasnoperekopsk District, "Red" Collective Farm	Chestnut, solonetz-like	Market garden	October	0–20 35–45	800 20	Irrigated area
Dzhankoi District, Novodzhankoi Vine Collective Farm	Dark chestnut	Vineyard	May	25–45 75–95	50 0	Unirrigated area

Table 2. Occurrence of Azotobacter in Southern Chernozems of the Central Steppe Zone

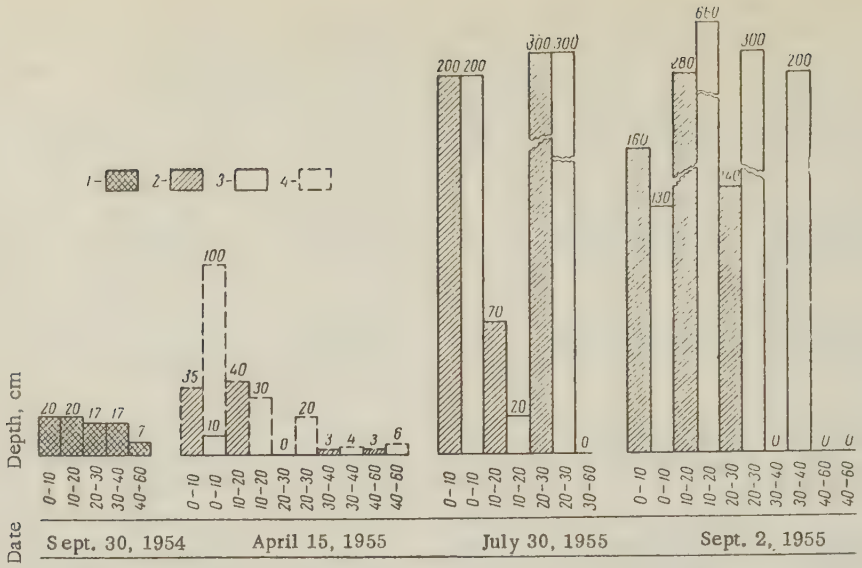
Sampling site	Crop	Time of sampling	Depth of sampling, cm	Numbers of Azotobacter in 1 g of soil	Notes
Krasnogvardeiskoe District, "Bolshevik" Collective Farm	Winter wheat on stubble	June	0–5 10–15 20–25	150 50 10	
Krasnogvardeiskoe District, "Bolshevik" Collective Farm	Winter wheat on fallow	June	0–5 10–15 20–25	60 70 40	
Krasnogvardeiskoe District, "Bolshevik" Collective Farm	Black fallow after cereals	June	0–5 10–15 20–25	100 Diffuse growth 10	
Krasnogvardeiskoe District, "Bolshevik" Collective Farm	Second-year alfalfa	June	0–5 10–15 20–25	100 60 0	Very thin
Krasnogvardeiskoe District, "Bolshevik" Collective Farm	Sainfoin sown in summer of previous year	June	0–5 10–15 20–25	100 300 Diffuse growth	Good stand, in flower
Krasnogvardeiskoe District, "Bolshevik" Collective Farm	Harrowed stubble	October	0–20 20–40 40–60	0 0 0	
Krasnogvardeiskoe District, "Bolshevik" Collective Farm	Long-fallow	October	0–20 20–40 40–60	0 0 0	
Krasnogvardeiskoe District, "Bolshevik" Collective Farm	Black fallow, plowed to depth of 40–50 cm without moldboard	October	0–20 20–40 40–60	20 10 0	
Field near auto highway 50 km north of Simferopol	Winter wheat	June	0–15 15–30 30–40	10 0 0	
Pervomaiskoe District, Chelyuskinets village	Pasture, virgin	November	0–20	0	
Pervomaiskoe District, Chelyuskinets village	Winter wheat	November	0–20	10	
Pervomaiskoe District, Chelyuskinets village	Black fallow Cotton	November	0–20 0–20	20 From 30 to 140	
Nizhnegorskii District, "Lenin" Collective Farm	Soil under tobacco crop	November	0–20 20–40	3500 180	Irrigated area

a depressed state. Mishustin (1954) refers to the possibility of such a temporary disappearance of Azotobacter.

The results of our investigations on southern chernozems of the central zone (Krasnogvardeiskoe, Pervomaiskoe, Nizhnegorskii Districts) are given in Table 2. These soils for the most part were poor in Azotobacter. Azotobacter was not found in the uncultivated soil here. It was practically absent from the fallow field, and was found in varying numbers under crops.

The foothill zone of the Crimea is distinguished by considerable diversity as regards relief, soil differences and moisture conditions, as well as by a great variety of cultivated crops. There are a considerable number of irrigated areas here. In this zone we studied carbonate chernozems on the higher ground, leached chernozems and meadow-chernozem soils in the valley of the Salgir River, which are not confined to this zone, but are found along the course of the river far beyond the limits of the zone.

In a previous work (Kolker, Dakhnova and Patenkov, 1958) we gave an account of the effects of autumn moldboardless plowing and plowing with moldboard on the microflora of carbonate and leached chernozems. Figure 1 gives the data on the occurrence of Azotobacter over the course of a year in the soil of fallow fields of the "Communar" Training Farm, where



Numbers of Azotobacter in the soil of a fallow field on the "Communar" training farm. 1)Before plowing; 2)autumn plowing; 3)spring plowing; 4)growth on soil plates.

autumn and spring moldboardless plowing to a depth of 36–40 cm was practiced.

Azotobacter occurred predominantly in the arable layer at depth 0–40 cm. In the 40–60-cm horizon their numbers were very small, and in most cases they could only be found on soil plates. It is clear from the figure that the numbers of Azotobacter were subject to fluctuation during the year. The greater activity of Azotobacter in late summer and early autumn was evidently due to the increased moisture in the soil and the moderate summer temperature in the year in which the observations were made. According to the figures obtained, spring plowing stimulated the development of Azotobacter more than autumn plowing.

Table 3 gives the data obtained from a study of similar soils in other farms.

As the table shows, the numbers of Azotobacter were very small in the unirrigated soils, which were mainly under field crops, but it was found in all the soil samples, especially in the upper horizons. Meadow-chernozem soils in the Salgir River valley, where most of the orchards, market gardens, and vineyards are situated, contained abundant Azotobacter (Table 3). It was found in varying numbers in every single soil sample.

The soils richest in Azotobacter were those of irrigated orchards ("Russia" Collective Farm, Simferopol District), especially old, long-cultivated soils. Azotobacter was also found in unirrigated soils, and in the carefully tilled orchards.

A typical feature of the valley meadow-chernozem soils was the occurrence of Azotobacter not only in the cultivated, but also in the uncultivated, soil of the meadows.

In the lower reaches of the Salgir we studied soils on the "Nizhnegorskii" Vine State Farm, Nizhnegorskii District. This state farm is situated at the confluence of two rivers—the Salgir and the Biyuk-Karasu. A typical feature of these soils was the high water table and the presence of a double humus horizon.

Table 4 gives the data on the occurrence of Azotobacter during the growing season on vineyard soils fertilized in different ways.

As we see from the figures in the table, Azotobacter was found in the upper layers of soil during the whole growing season. It was most abundant in September at the time when the vines were ripening. In November the numbers of Azotobacter cells dropped considerably.

In the lower horizons of the unfertilized soil the numbers of Azotobacter decreased with depth, and in the 60–70-cm layer it could no longer be found, whereas when fertilizer was applied Azotobacter was not only found at a considerable depth, but sometimes it was more numerous there than in the upper layer (the fertilizer was applied to a depth of 30–40 cm).

As we see from the figures in Table 4, organic and mineral fertilizers applied together had the most stimulating effect on the development of Azotobacter.

In the mountain zone of the Crimea, Azotobacter was found in very small numbers during the period of our observations, and only in samples of soil from Ai-Petri.

SUMMARY

1. Azotobacter occurs widely in the soils of the foothills and steppe zones of the Crimea, although it is sometimes present in very small, barely detectable numbers. The soils richest in Azotobacter are the meadow-chernozem soils of the Salgir River valley, and the soils poorest in Azotobacter are the southern chernozems of the steppe zone.

2. The Azotobacter content of the soil is subject to fluctuation depending on the season and moisture conditions. It is most abundant in spring and early summer. Where irrigation is practiced or where the summer rainfall is regular the Azotobacter content of the soil is still high in the autumn.

Table 3. Occurrence of Azotobacter in Chernozems of the Foothill Zone of the Crimea

Sampling site	Soil	Crop	Time of sampling	Depth of sampling, cm	Numbers of Azotobacter	Notes
Simferopol District, "Rebirth" Training Farm	Carbonate chernozem	Sward of perennial grasses	January	5–10 30–35	42 0	Unirrigated land
Simferopol District, "Rebirth" Training Farm	Carbonate chernozem	Winter wheat on a three-year sward of alfalfa	September	0–20	From 30 to 70	Unirrigated land
Simferopol District, "Rebirth" Training Farm	Carbonate chernozem	Field after autumn plowing to depth 40–42 cm	July	0–20 20–60	35	Unirrigated land
Simferopol District, "Rebirth" Training Farm	Carbonate chernozem	Field after autumn plowing to depth 40–42 cm	July	0–10 10–20 20–60	170 12 0	Unirrigated land
Simferopol District, Zhdanov Collective Farm	Leached chernozem	Field under sunflower after cultivation	September	0–10 10–20 20–40 40–60	200 80 30 2	Unirrigated land
Simferopol District, "Russia" Collective Farm	Leached chernozem	Winter wheat on early fallow	June	0–20	From 230 to 940	Unirrigated land
Salgir River flood-plain, near Pionerskoe village	Meadow-chernozem	Meadow	October	0–20	160	
Salgir River flood-plain, near Kakhovskoe village	Meadow-chernozem	Meadow	June	0–20	1000	
Pionerskoe village	Meadow-chernozem	Orchard	October	0–20	2000	Irrigated
Kaskhovskoe village, left bank	Meadow-chernozem	Old orchard	June	0–20	4500	Irrigated
Kakhovskoe village, left bank	Meadow-chernozem	Orchards, turfed between trees; pear, apple	June	0–20 0–20	860 2400	Irrigated
Kakhovskoe village, left bank	Meadow-chernozem	Apple orchard, bare fallow	June	0–20	2500	Unirrigated
"Russia" Collective Farm, right bank of Salgir	Meadow-chernozem	Apple orchard, intercrop of alfalfa	June	0–20	1620	Irrigated
"Russia" Collective Farm, right bank of Salgir	Meadow-chernozem	Young apple orchard	June	0–20	8500	Irrigated
"Russia" Collective Farm, right bank of Salgir	Meadow-chernozem	Old apple orchard	June	0–20	30000	Irrigated

Table 3 (Continued)

Sampling site	Soil	Crop	Time of sampling	Depth of sampling, cm	Numbers of Azotobacter	Notes
"Russia" Collective Farm, right bank of Salgir	Meadow-chernozem	Old cherry orchard	June	0–20	From 28,000 to 42,000	Irrigated

Table 4. Numbers of Azotobacter in Irrigated Vineyard Soil Fertilized in Different Ways

Sampling site	Depth of horizon, cm	Numbers of Azotobacter in 1 g of soil					
		May	June	July	August	Sept.	Nov.
Unfertilized vineyard (control)	5—15	290	100	80	20	595	40
	30—40	50	20	70	10	50	0
	60—70	0	0	0	0	0	0
Vineyard fertilized with 25 T manure per ha	5—15	675	60	110	240	330	10
	30—40	270	10	340	250	970	20
	60—70	30	10	20	80	130	50
Vineyard fertilized with 5 T manure + NPK	5—15	330	380	620	800	1030	110
	30—40	775	825	180	100	760	0
	60—70	360	470	750	0	1000	40
Vineyard fertilized with 5 T manure + NP	5—15	400	480	300	300	950	400
	30—40	560	410	100	150	3000	700
	60—70	20	130	580	180	1350	0
Vineyard fertilized with 5 T manure + N	5—15	450	570	1200	240	780	20
	30—40	1240	550	150	120	1220	20
	60—70	150	10	300	50	720	0
Vineyard fertilized with NPK	5—15	480	20	90	50	140	10
	30—40	50	50	20	40	370	20
	60—70	0	20	0	0	0	0

3. The plant cover and degree of cultivation of the soil affects the occurrence of Azotobacter. It is least abundant in uncultivated soils, and under cereal crops. Vines, tobacco, and alfalfa have a beneficial effect on it. Congenial conditions for Azotobacter are provided in well-tilled and irrigated orchards.

4. The application of organic, and particulary of organic and mineral fertilizers together, stimulates the development of Azotobacter.

5. According to their morphological and cultural features, Azotobacter strains isolated from different soils of the Crimea can be assigned to the species Azotobacter chroococcum.

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ACTINOMYCETES FROM SOME SOILS OF THE PAMIRS AND THEIR ANTAGONISTIC PROPERTIES

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The collection of data on the occurrence of actinomycetes in different soils of the USSR is not only of theoretical interest, but is of practical interest too, in connection with the research being done on producers of new antibiotics.

The occurrence of actinomycetes in soils of the USSR has been the subject of several works by Soviet authors (Krasil'nikov, 1950; Mishustin and Mirzoeva, 1953; Panosyan and Tumanyan, 1953; Korenyako, et al., 1955; Egorov and Polin, 1955; Teplyakova and Maksimova, 1957; Zharikova, et al., 1958; Kuznetsov, 1958, 1959; Solov'eva and Taig, 1959; and others). Nevertheless, we still know very little about the occurrence of actinomycetes in the soils of the USSR, particularly of remote and inaccessible regions.

The present paper gives the results of a study of the species (group) composition and of tests of the antagonistic properties of actinomycetes isolated from some soils of inaccessible regions of the Pamirs.

The soil samples for the investigation were given to us by N. N. Tsvelev, a member of the staff of the Institute of Botany, Academy of Sciences, USSR, who had kindly agreed to undertake the collection of soil samples.

METHODS

Twenty-four soil samples collected in 1958 at high altitudes on the Pamirs were investigated. The soil samples were collected by aseptic technique among the roots of plants at depths down to 10 cm. Each soil sample was plated out three times on SR-1 medium containing glucose (Krasil'nikov, 1950). The colonies were counted and the actinomycetes isolated after 6 days' growth at 26°. The actinomycetes were identified by the usual methods. The antimicrobial spectrum was determined by the agar block method after the isolated actinomycetes had been cultivated for 14 days on two agar media—maize and fish. The composition of the maize medium was: maize extract—10 g (dry weight), calcium carbonate—5 g, ammonium sulfate—3.5 g, sodium chloride—5 g, glucose—10 g, starch—15 g, agar—20 g, tap water—1 liter. The fish medium had the following composition: fish extract—15 g, glucose—20 g, calcium carbonate—5 g, agar—20 g, tap water—1 liter. The test microbes used were Staphylococcus aureus 209, Escherichia coli, Candida albicans, Mycobacterium sp. B-5, Bacillus mycoides 1104, and Thieliviopsis basicola.

EXPERIMENTAL

The data on the sampling sites, vegetation, and numbers of actinomycetes isolated are given in Table 1. A striking feature was the presence of a relatively large number of representatives of the genus Proactinomyces (70 strains out of 933 isolated cultures) in the investigated soil samples.

It was not possible to identify all the isolated cultures accurately. Some of the cultures were identified to species or aggregate (group) species. We divided the remaining cultures provisionally into two groups according to the color of their substrate mycelium. The color of the mycelium was determined from Bondartsev's color scale (1954).

Table 2 gives the data on the distribution of individual soil samples.

As we see from Table 2, in some soil samples (Nos. 1, 2, 7, 10) representatives of the genus Proactinomyces were also found among the dominant groups. In the remaining samples the dominant actinomycetes belonged to the groups: A. griseus (9), A. levoris (6, 13), A. lavendulae (11, 14, 15), A. fasciculus (12), A. verticillatus (16, 17), A. albus sterilis (18), A. rectus bruneus (20), A. globisporus (21), A. globosus (22, 23), A. fradii (25), and unidentified actinomycetes with substrate mycelium colored yellow (5), dark smoky (15), or brown (24).

Among all the isolated cultures the greatest numbers of actinomycetes belonged to the groups A. verticillatus (20.6%), A. levoris (16%), and A. fradii (10.1%) (Table 3).

As Table 3 shows, besides the actinomycetes mentioned already, the investigated soils contained considerable numbers of representatives of A. albus sterilis, A. candidus, A. globisporus, A. griseus, and A. lavendulae, as well as of actinomycetes with substrate mycelium colored brown, yellow, olive-grey, or dark umber. Representatives of A. chromogenes, A. coremiales, A. fasciculus, A. flavus, A. graminearum, A. griseolus, A. rectus bruneus, A. violaceus chromogenes, and A. vulgaris were present only in isolated cases.

Besides actinomycetes, we isolated from the Pamir soils a relatively large number of cultures of the genus Proactinomyces, closely related to P. actinomorphus—P. albus, P. flavescens, P. flavus, and others.

A comparative study of the antagonistic properties of the isolated cultures grown on maize and fish media

Table 1. Sampling Sites and Numbers of Isolated Actinomycetes from Pamir Soils

No. of soil sample	Sampling site	Vegetation	Number of isolated representatives of		Total
			genus Actino-myces	genus Proactino-myces	
1	Pamir Biological Station. Chechekati, near Osh-Murgab road. Height 3850 m	Artemisia skornjakovii	15	7	22
2		Ranunculus sp. and grasses	6	5	11
3		Eurotia ceratoides	0	0	0
4		Elymus sibiricus	3	0	3
5		Carex sp., Astragalus heterodontus	77	17	94
6	R. Murgab valley, 3 km west of confluence of Zap. Pshart River. Height 3320 m	Elymus dasystachys	109	3	112
7		Astragalus heterodontus	12	16	28
8		Eurotia ceratoides	0	0	0
9		Hippophae rhamnoides	7	0	7
10		Artemisia dracunculus	9	10	19
11	Ak-Tash Pass. Height 4300 m	Carex pseudofoetida, Kobresia pamiroalaica	2	0	2
12		Oxytropis melanotricha	12	0	12
13		Hordeum turkestanicum, Poa litwinowii	71	1	72
14		Smelowskia calycina	27	10	37
15		Acantholimon diapensioides	59	1	60
16	6 km north of Takhta-Korum Pass. Height 4300 m	Kobresia capilliformis, Carex melananthae	170	0	170
17		Hedysarum cephalotes	59	0	59
18	Takhta-Korum Pass. Height 4500 m	Puccinellia humilis	28	0	28
20		Rhodiola gelida	5	0	5
21		Oxytropis melanotricha	18	0	18
22		Nepeta pamirica	8	0	8
23		Kobresia persica, Erigeron heterochaetus, Leontopodiumoch ochroleucum	17	0	17
24	Left bank of Takhta-Korum River, 4 km from Takhta-Korum Pass, Height 3900 m.	Artemisia tianschanica	44	0	44
25		Stipa orientalis	105	0	105
		Total isolated	836 (92.5%)	70 (7.5%)	933 (100%)

Table 2. Actinomycetes Found in Investigated Pamir Soils

No. of soil sample	Groups and species	Dominant groups and species
1	Act. globisporus, Act. levoris, Act. vulgaris, olive-grey actinomycetes, Proact. actinomorphus, Proact. flavescens	Act. vulgaris, Proact. flavescens
2	Act. globisporus, olive-grey actinomycetes, Proact. flavescens, Proact. flavus	Olive-grey actinomycetes, Proact. flavescens
4	Act. lavendulae, Act. griseolus, olive-grey actinomycetes	
5	Act. globisporus, olive-grey and yellow actinomycetes	Yellow actinomycetes
6	Act. levoris, yellow and dark umber actinomycetes, Proact. flavescens, Proact. flavus	Act. levoris
7	Act. levendulae, yellow and colorless actinomycetes, Proact. albus	Proact. albus
9	Act. griseus, Act. lavendulae, brown actinomycetes	Act. griseus
10	Act. lavendulae, olive-grey actinomycetes, reddish-brown and dark smoky proactinomycetes	Reddish brown proactinomycetes
11	Act. lavendulae	Act. lavendulae
12	Act. albus sterilis, Act. fasciculus, Act. globisporus, Act. levoris, yellow actinomycetes	Act. fasciculus
13	Act. albus, sterilis, Act. griseus, Act. lavendulae, Act. levoris, yellow, dark umber actinomycetes, Proact. albus	Act. levoris
14	Act. chromogenes, Act. flavus, Act. globisporus, Act. globosus, Act. lavendulae, Act. levoris, Act. graminearum, olive-grey and yellow actinomycetes, Proact. flavescens, Proact. flavus	Act. lavendulae
15	Act. candidus, Act. fradii, Act. globisporus, Act. globosus, Act. lavendulae, olive-grey, yellow, dark umber and black actinomycetes, dark smoky proactinomycetes	Act. lavendulae
16	Act. globisporus, Act. levoris, Act. verticillatus, yellow, dark umber actinomycetes	Act. verticillatus
17	Act. albus sterilis, Act. candidus, Act. verticillatus, olive-grey and brown actinomycetes	Act. verticillatus
18	Act. albus sterilis	Act. albus sterilis
20	Act. lavendulae, Act. rectus bruneus	Act. rectus bruneus
21	Act. globisporus, Act. lavendulae, brown actinomycetes	Act. globisporus
22	Act. globisporus, Act. globosus, brown actinomycetes	Act. globosus
23	Act. globisporus, Act. globosus, Act. lavendulae	Act. globosus
24	Act. albus sterilis, Act. candidus, Act. globisporus, Act. globosus, Act. griseus, Act. lavendulae, Act. rectus bruneus, yellow, brown and colorless actinomycetes	Brown actinomycetes
25	Act. albus sterilis, Act. coremiales, Act. fradii, Act. violaceus chromogenes	Act. fradii

Table 3. Quantitative Relationships of Different Groups of Actinomycetes Isolated from Pamir Soils

Groups and species	No.	%	Groups and species	No.	%
Act. albus sterilis	39	4,1	Act. violaceus chromogenes	2	0.2
Act. candidus	12	1,2	Act. vulgaris	6	0.6
Act. chromogenes	1	0,1	Colorless	8	0,8
Act. coremiales	9	0,9	Brown	19	2.0
Act. fasciculus	4	0,4	Yellow	69	7.3
Act. flavus	1	0,1	Olive-gray	34	3.6
Act. fradii	95	10,1	Dark umber	34	3.6
Act. globisporus	70	7,5	Black	3	0.3
Aci. globosus	28	3,0	Proact. actinomorphus	1	0.1
Act. graminearum	1	0,1	Proact. albus	17	1.8
Act. griseolus	1	0,1	Proact. flavescens	35	3.7
Act. griseus	13	1,3	Proact. flavus	6	0.6
Act. lavendulae	66	7,0	Reddish brown	9	0,9
Act. levoris	150	16,0	Dark smoky	2	0.2
Act. rectus bruneus	5	0,5	Total	933	100
Act. verticillatus	193	20,6			

Table 4. Manifestation of Antibiotic Activity by Actinomycete Cultures Grown on Maize and Fish Media

Medium	Total no. of cultures tested	Those inhibiting growth of											
		Staph. aureus 209		E. coli		Candida albicans		Mycobact. sp. B-5		Bac. mycoides 1104		Thieliviopsis basicola	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Maize	933	483	51.7	358	38.3	353	37.8	414	44.3	543	58.1	394	42,1
Fish	933	541	57.9	406	43.4	311	33.2	364	38.9	440	47.1	339	36.2

Table 5. Antimicrobial Spectrum of Different Groups of Actinomycetes Isolated from Pamir Soils

Groups and species	Total no. of cultures isolated	Those inhibiting growth of											
		Staph. aureus 209		E. coli		Candida albicans		Mycobact. B-5		Bac. mycoides		Thieliviopsis bisicola	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Act. albus sterilis	39	2	5.0	1	2.5	1	2.5	1	2.5	0	0	0	0
Act. candidus	12	7	58.3	2	16.6	2	16.6	5	41.6	11	91.6	2	16.6
Act. chromogenes	1	1	100	1	100	0	0	1	100	1	100	0	0
Act. coremiales	9	8	88.8	0	0	0	0	0	0	4	44.4	0	0
Act. fasciculus	4	0	0	0	0	0	0	0	0	0	0	0	0
Act. flavus	1	0	0	0	0	0	0	0	0	0	0	0	0
Act. fradii	95	22	23.1	10	10.5	0	0	2	2.1	45	47.3	0	0
Act. globisporus	70	59	84.2	37	52.8	4	5.7	29	41.4	30	42.8	45	64.2
Act. globosus	28	8	28.5	6	21.4	7	25.0	6	21.4	6	21.4	0	0
Act. graminearum	1	1	100	1	100	0	0	1	100	1	100	0	0
Act. griseolus	1	1	100	0	0	0	0	0	0	1	100	0	0
Act. griseus	13	7	53.8	7	53.8	1	7.6	8	61.5	6	46.1	5	38.4
Act. lavendulae	66	60	90.9	42	63.6	8	12.1	24	36.3	66	100	8	12.1
Act. levoris	150	17	11.3	2	1.3	124	82.0	107	71.3	122	83.3	64	42.6
Act. rectus bruneus	5	0	0	0	0	0	0	0	0	0	0	0	0
Act. verticillatus	193	193	100	193	100	193	100	193	100	193	100	193	100
Act. violaceus chromogenes	2	0	0	0	0	0	0	0	0	2	100	0	0
Act. vulgaris	6	5	83.3	0	0	0	0	1	16.6	5	83.3	5	83.2
Colorless	8	0	0	0	0	4	50.0	0	0	0	0	0	0
Brown	19	5	26.3	4	21.0	0	0	2	10.5	5	26.3	0	0
Yellow	69	54	78.3	30	43.4	7	10.1	15	21.7	21	30.4	48	69.5
Olive-gray	34	6	17.6	3	8.8	1	2.9	1	2,9	2	5.8	6	17.6
Dark umber	34	19	55.8	11	32.3	0	0	15	44.1	18	52.9	0	0
Black	3	0	0	0	0	0	0	0	0	0	0	0	0
Proact. actinomorphus	1	0	0	0	0	0	0	0	0	0	0	0	0
Proact. albus	17	0	0	0	0	0	0	0	0	0	0	5	29.4
Proact. flavescens	35	6	17.1	8	22.8	1	2.8	3	8.5	0	0	1	2.8
Proact. flavus	6	2	33.3	0	0	0	0	0	0	2	33.3	2	33.3
Reddish brown	9	0	0	0	0	0	0	0	0	2	22.2	9	100
Dark smoky	2	0	0	0	0	0	0	0	0	0	0	1	50.0

Table 6. Characteristics of Verticillate Actinomycetes Isolated From Pamir Soils Compared with Those of Actinomyces verticillatus (Kriss, 1938)

Taxonomic characters	Verticillate actinomycetes from Pamir soils	Act. verticillatus (Kriss, 1938)
Sporophores	Slightly bent, arranged in primary whorls	Slightly bent, arranged in primary whorls
Spores	Elongate with rounded ends	Cylindrical or elongate with rounded ends
Growth on synthetic medium (Czapek)	Substrate mycelium brown, secreting a brown substance into medium. Aerial mycelium fluffy, whitish pink	Substrate mycelium colorless or slightly brownish. Aerial mycelium velvety or fluffy, white at first, later grey or greyish green
Liquefaction of gelatin	Do not liquefy	Liquefies rapidly
Effect on milk	No coagulation, peptonization starts on 10th day	Coagulation and peptonization
Reduction of nitrates	Do not reduce	No information
Inversion of saccharose	Invert weakly	Inverts
Hydrolysis of starch	Hydrolyze weakly	Hydrolyzes
Growth on cellulose	No growth	No growth
Inhibits growth of	Staph. aureus Bact. coli Candida albicans Mycobacterium sp. B-5 Thieliviopsis basicola Bact. mycoides	No information

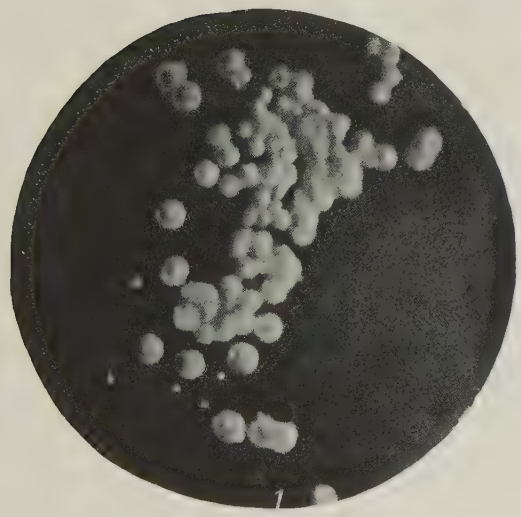

Fig. 1. Two-day old colonies of actinomycete No. 17–41 on Czapek medium containing 1% starch. Natural size.

Fig. 2. Structure of sporophores in actinomycete No. 17–41 grown on Czapek medium containing 1% starch Mag. 100 x.

showed that these two media were practically of equal value for revealing the antagonistic properties of actinomycetes (Table 4), although in some cases one of the media was better for revealing antagonism in individual representatives of the groups of actinomycetes. For instance, the antagonistic properties of many strains of the A. globisporus, yellow actinomycetes and A. levoris groups were manifested more clearly on the maize medium, and those of A. vulgaris and A. albus sterilis on fish medium.

Table 5 shows the antimicrobial spectrum of the different groups of isolated actinomycetes. It is clear from Table 5 that all the 193 strains of actinomycetes closely related to A. verticillatus inhibited the growth of all the test-microbes used in the investigations. A great number of antagonists were also found in the A. globisporus, A. candidus, A. coremiales, and A. lavendulae groups, as well as among actinomycetes where the substrate mycelium was of yellow or olive-grey color. Few antagonists were found amoung the representatives of the genus Proactinomyces.

Of the antagonists which we found among all the isolated representatives of the genera Actinomyces and Proactinomyces 57.9% were active against S. aureus, 43.4% against E. coli, 37.8% against C. albicans, 44.3% against M. sp. B-5, 58.1% against B. mycoides, and 42.1% against T. basicola.

From the soil samples Nos. 16 and 17 we isolated 193 identical strains of actinomycetes, closely related morphologically to A. verticillatus (Krasil'nikov, 1949), but differing from this species in their cul-

tural and physiological characters (Table 6 and Figs. 1 and 2).

We see from Table 6 that the verticillate actinomycetes isolated from Pamir soils formed whitish pink aerial mycelium when grown on synthetic medium, and that old cultures did not produce the dark grey or greyish green mycelium typical of A. verticillatus. Moreover, the isolated strains, as distinct from A. verticillatus, did not liquefy gelatin and did not coagulate milk.

From the mycelium of the verticillate strain No. 17-41 we obtained a mixture of polyene antibiotics in the form of an ethanol extract. From preliminary results of determining the maxima of light absorption, the ethanol extract of the mycelium of strain No. 17-41 contained at least two antibiotics, belonging to the pentaene and heptaene groups.

The author expresses his deep thanks to N. N. Tsvelev of the Institute for Botany for collecting the soil samples and identifying the plants under which the soil samples were collected, and also to his colleague V. B. Korchagin in the VNIIA for determining the nature of the antibiotics produced by strain No. 17-41.

SUMMARY

1. The species (group) composition of actinomycetes from some samples of alpine soils from the Pamirs was studied.

2. In individual soil samples the following groups of the genus Actinomyces predominated: A. albus sterilis, A. fasciculus, A. fradii, A. globisporus, A. globosus, A. griseus, A. lavendulae, A. levoris, A. rectus bruneus, A. verticillatus, A. vulgaris, and also unidentified cultures in which the substrate mycelium was brown, yellow, or olive-grey.

3. From the Pamir soil samples a fairly large number of representatives of the genus Proactinomyces were isolated. Out of the 933 cultures isolated from the investigated soil samples 70 strains belonged to the genus Proactinomyces.

4. From soil samples taken near the Takhta-Korum Pass at a height of 4300 m, 193 identical strains of actinomycetes, similar in morphology to A. verticillatus, were isolated. According to preliminary results, these cultures produce a mixture of polyene antibiotics belonging to the group of heptaenes and pentaenes.

5. The numbers of actinomycetes which are antagonistic towards the test-microbes used in the work were as follows: 57.9% of all the isolated cultures of actinomycetes and proactinomycetes were antagonistic to Staphylococcus aureus, 43.4% to Escherichia coli, 37.8% to Candida albicans, 44.3% to Mycobacterium sp. B-5, 58.1% to Bacillus mycoides, and 42.1% to Thieliviopsis basicola.

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PHAGOLYSIS OF BACILLUS MEGATERIUM IN THE PRODUCTION OF PHOSPHOROBACTERIN

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Menkina (1950) has described bacteria she isolated which were capable of decomposing organic phosphorus compounds. The most active of these was a strain of Bacillus megaterium which she referred to as B. megaterium var. phosphaticum.

Menkina showed that the introduction of these bacteria into soil in the form of the pure culture or of the bacterial preparation called phosphorobacterin increases the crop yield of agricultural plants.

Phosphorobacterin, which was tested by a series of investigators, has been widely accepted in agricultural practice and has begun to be manufactured in several industrial bacteriological laboratories.

Phosphorobacterin is prepared by means of a depth method. At the First State Industrial Bacteriological Laboratory a periodic mass phagolysis of the industrial culture was noted (beginning at the end of 1955).

Lysis was observed in inoculators in which the seeding material was prepared, but most often it occurred in the reactors in which the mass industrial cultures were grown. In some cases all the bacterial mass was lysed; in other cases the quantity of bacterial cells dropped at various stages. Phagolysis also occurred at the Kiev Industrial Bacteriological Laboratory which manufactures phosphorobacterin by the same method.

The present article is devoted to an account of some of the results of the study of phagolysis at the First State Industrial Bacteriological Laboratory, where these observations were made in the course of 1956–1959.

EXPERIMENTAL DATA

In 1955, and during the years following, the No. 2 strain of B. megaterium var. phosphaticum developed by Menkina (1950) and several variants of this strain which she selected were adapted for cultivation at the industrial laboratory.

Strain No. 2 and its variants presented a typical picture of B. megaterium in both cultural and morphological properties.

It is known that the lysis of a microbial cell can be caused not only by a bacteriophage, but also by various other factors, among which so-called bacteriocin is of special interest.

Ivanovics and Alföldi (1954) showed that the B. megaterium strain 216, when cultivated on a yeast medium with hydrolysate of casein, produces a peculiar substance which they called megacin. This substance possessed antibacterial properties both against its own culture and against all other strains of B. megaterium which they tested. In subsequent works (1955, 1957) the same authors presented much interesting data on megacin. It appeared that the formation of megacin occurs as the result of lethal biosynthesis and coincides with the moment of cell lysis. Ultraviolet-irradiation of the cultures of B. megaterium capable of producing megacin caused lysis of the cells with the release of megacin into the medium. In this case, however, no phage was revealed in the lysate. Induced formation of megacin takes place best on complex organic media and has not been observed on synthetics.

In order to discover the fundamental cause of lysis of the B. megaterium culture under industrial conditions, we first undertook to demonstrate the presence of specific bacteriophages in the lysed material.

For this purpose we passed through a Zeitz filter the culture liquid from the series of inoculators and reactors in which the bacterial mass was known to have been lysed, and determined the presence of bacteriophages in the filtrates.

In view of the fact that in the initial period of the appearance of mass lysis at the bacteriological laboratory the culture of B. megaterium var. phosphaticum, strain No. 2, was being used, the presence of bacteriophage in the filtrates was therefore determined in reference to this culture when it was grown on the solid and liquid media used in the preparation of phosphorobacterin at the laboratory. The composition of the medium was as follows: molasses, 0.6%; corn extract, 0.6%; $(NH_4)_2SO_4$, 0.05%; $MgSO_4$, 0.03%; KCl, 0.03%; NaCl, 0.03%; calcium carbonate, 0.5%. We put the tested filtrates into the retort at the same time as the inoculum of the No. 2 culture. The retorts were left to mature on a rocker at 27–28° for 24 hours.

In not one of the numerous experiments did we observe lysis of the No. 2 culture by the filtrates, and the growth in the retorts with the filtrates did not differ from the growth in the control retorts (without filtrates.)

In most of the experiments with the addition of the filtrates to freshly prepared seedings of the No. 2

culture on medium solidified with agar no lysis of the culture was noted. Only in individual experiments on a seeding of No. 2 culture did there appear small plaques with absence of growth of the culture at those places where the filtrates had been added. However, attempts to obtain bacteriophage from these plaques and to reproduce it in No. 2 culture were unsuccessful.

In order to determine whether this culture liquid contained bacteriophages able to lyse other cultures of B. megaterium, the series of filtrates were examined using test cultures of variants of No. 2 selected by Menkina (strains from January 19, 1956, and January 28, 1956, designated by us as PSA and PSB respectively), and, besides these, of a series of typical cultures of B. megaterium from the museum of the Institute of Microbiology, Academy of Science, USSR.

In Table 1 are presented the results demonstrating the presence of bacteriophages in six filtrates of the No. 2 culture liquid which had undergone lysing in the inoculators or reactors.

From the data of Table 1 it can be seen that in all six of the filtrates tested there were bacteriophages present which were capable of lysing the variants of the No. 2 culture (strains PSA and PSB) and the museum culture of No. 7. The culture of B. megaterium var. phosphaticum, strain No. 2, appeared resistant to these bacteriophages. With titration by the agar-overlay method on cultures PSA and PSB the titer of the phage in the lysed culture liquid was found to be from 10^7 to 10^{10} and greater. Most often the titer was an even 10^9–10^{10}.

Later on in the course of 1956 and 1957 when phagolysis of the No. 2 culture occurred under industrial conditions, many times we obtained bacteriophages from the lysed material which were active against strains PSA and PSB but did not lyse the No. 2 culture.

The following could be the only explanation for this phenomenon: The culture of B. megaterium var. phosphaticum, strain No. 2, appears to be lysogenic and therefore it is resistant to the bacteriophage it carries. This culture is able to engender phage-susceptible mutants. A similar phenomenon has been described in other lysogenic cultures, in particular by Stolp in B. megaterium (1957, 1958).

According to the data of the present author, the frequency rate in the appearance of phage-susceptible mutants in his tests was $(4.93 \pm 0.17) \times 10^{-8}$ cells. Stolp (1958) notes that it is possible for reverse mutation of B. megaterium to take place, from phage-susceptibility to phage-resistance.

The phage-resistant culture in Stolp's experiments formed R-type colonies, while the phage-susceptible cultures formed S-type colonies.

In our tests no distinctions among the colony types and the host-phage relationships were noted. The No. 2 culture and its phage-susceptible mutants were culturally indistinguishable and all formed S-type colonies.

The cultures obtained by seeding the culture liquid of strain No. 2 which had been lysed under industrial conditions as a rule were resistant to the phages obtained from this same liquid.

In order to clear up the question of the causes for the phagolysis of No. 2 culture we set up a series of experiments to study the formation by this culture of phage-susceptible mutants.

For this purpose we investigated the phage-susceptibility of cultures taken from individual colonies grown from a seeding of the laboratory's source strain of No. 2. The material for seeding was taken from the culture grown on a liquid medium in inoculators and reactors under industrial conditions, as well as from the culture grown on medium solidified with agar.

Susceptibility of the cultures to the phages was determined by adding them to a freshly prepared seeding on PCA (peptone-corn agar)medium with cultivation at 27–28°.

Table 1. Results of Several Experiments to Demonstrate the Presence of Bacteriophages in Filtrates of the Culture Liquid from the Lysed Bacillus megaterium Culture

	Filtrates					
	from inoculator 1/20/56	from inoculator 1/28/56	from inoculator 1/27/56	from reactor 1/7/56	from reactor 1/7/56	from reactor 2/6/56
B . megaterium var. phosphaticum, strain No. 2	–	–	–	–	–	–
The same, variant PSA from strain No. 2	+	+	+	+	+	+
The same, variant PSB from strain No. 2	+	+	+	+	+	+
B . megaterium, museum strain No. 2 from the Institute of Microbiology	–	–	–	–	–	–
The same, strain No. 6	–	–	–	–	–	–
The same, strain No. 7	+	+	+	+	+	+
The same, strain No. 9	–	–	–	–	–	–
The same, strain No. 10	–	–	–	–	–	–
The same, strain No. 11	–	–	–	–	–	–
The same, strain No. 12	–	–	–	–	–	–
The same, strain No. 15	–	–	–	–	–	–

Note. + indicates presence, – indicates absence in the filtrate of bacteriophage capable of lysing the test culture.

These tests, which were repeated frequently, were conducted both at the industrial laboratory and at the Institute of Microbiology.

We present here the results of several of these experiments. The seeding of the agar culture of No. 2 was carried out on MPA (meat-peptone agar) and 106 cultures were made from the separate colonies. Tests on the susceptibility of these 106 cultures to three bacteriophages (Nos. 1, 9, and 16) isolated at the laboratory at the time of the phagolysis of No. 2 culture showed that 73 cultures were resistant to all three phages, 33 cultures were susceptible to phages 1 and 16, and 29 cultures were susceptible to phage 9. The percentage of phage-susceptible variants was not the same in all tests with inoculum of the culture from a medium solidified with agar.

In some tests the percentage of phage-susceptible cells was significantly lower. For example, in one group of 79 colonies, 16 were phage-susceptible. There also were tests in which all the individual colonies made with a seeding of agar culture were phage-resistant. Thus, in the second seeding of a phage-resistant variant of No. 2 culture, 60 cultures were made on MPA and 60 cultures were made on PCA. All these 120 cultures proved to be phage-resistant. It is important to emphasize that phage-resistant variants set apart during seeding of the No. 2 culture also engendered phage-susceptible cultures in the next seeding. The property of engendering phage-susceptible mutants is linked with the lysogenic composition of the culture, and, apparently, is a result of the unequal division of the lysogenic cells by which the prophage either remains in the mother cell or passes into a daughter cell so that one of the cells ceases to be lysogenic and thereby is rendered susceptible to the phage carried by the original parent cell.

The phage-susceptible mutants of No. 2 culture do not differ from the original strain either culturally or morphologically, and therefore it is possible to distinguish the one from the other only by examination of their host-phage relationships.

It is quite possible that in the seeding of the No. 2 culture, separate colonies could germinate from two cells closely attached to each other, one of which is phage-resistant, while the other is phage-susceptible. It is obvious that such a mixed culture would contain a large quantity of phage-susceptible cells. It was established afterwards that this occurred in a series of explantings of strain No. 2 obtained during serial transfers, several of which consisted only of phage-susceptible cells.

Experiments to determine the quantity of phage-susceptible cells which were formed by the No. 2 culture under cultivation in the inoculators and reactors showed that in a series of cases this could be rather high. In some tests the quantity of susceptible cells amounted to 20–40% and in others it was equivalent to 70–75% and higher. Undoubtedly the composition of the original seeding culture greatly influenced the quantity of phage-susceptible cells which germinated in the No. 2 culture under cultivation in the inoculators and reactors.

The data here presented show that the culture of B. megaterium var. phosphaticum, strain No. 2, freely produced phage-susceptible cells when cultivated on liquid and solid media. The quantity of these mutants can vary, depending on both the composition of the original culture and, apparently, the conditions of cultivation.

During the cultivation of a lysogenic culture, usually a determinate quantity of the cells are lysed, releasing active particles of the phage carried by the given culture. Lwoff (1953) notes that in the lysogenic cultures of B. megaterium under the usual conditions of cultivation approximately one out of 100,000 cells is lysed, thereby releasing 100 particles of active phage.

Under laboratory conditions of cultivation isolation of the phage contained within the culture of B. megaterium var. phosphaticum, strain No. 2, is rarely accomplished, and then only with great difficulty. Once, we succeeded in isolating phage when this culture was grown together with a culture susceptible to the phage which it carries. Additional attempts to isolate the phage ended in failure. The attempts by N. Ya. Solovyova to induce UV phage in the No. 2 culture also yielded negative results. It is quite possible that strain No. 2 is one of the noninducible cultures.

On the basis of these results we have arrived at the conclusion that in this series of cases the cause of phagolysis of the culture of B. megaterium var. phosphaticum, strain No. 2, was its lysogenicity and ability to engender phage-susceptible mutants comparatively freely. The phage-susceptible mutants engendered during the growth of this culture are lysed as a result of interaction with the phage it is carrying.

The degree of lysis of the culture under industrial conditions depends on the number of phage-susceptible mutants which are engendered and on the number of active particles of phage which arise with the "spontaneous" lysis of part of the cells. Lysis of an insignificant number of cells with the release of active particles of phage occurs rather frequently. This is shown by data from experiments demonstrating the presence of phage in the culture liquid from reactors of those fermentations in which the growth of the culture was considered normal according to microscopic findings and composites (the number of viable cells), i.e., it satisfied the technical requirements for the manufacture of phosphorobacterin and for the end product itself.

Phage capable of lysing PSB culture was revealed in 30 of the 71 specimens of liquid phosphorobacterin investigated. Phage was revealed in six specimens of liquid phosphorobacterin after storage for 15 months in the warehouse. The titer of the phage in several specimens of the final liquid phosphorobacterin reached 10^6 and higher.

Phage was repeatedly revealed in the dry phosphorobacterin. Thus, out of three series of dry phosphorobacterin prepared in 1956, phage was revealed in two (series 47 and 87) in large quantity, the titer for 1 g of phosophorobacterin being greater than 10^8 in series 47 and 10^6 in series 87. After four months of storage

these series of dry phosphorobacterin were examined by Dorosinskii and Kvaratskheliya (1958) who demonstrated the presence in them of approximately the same quantity of phage. These data indicate the relative resistance of the B. megaterium phage throughout dehydration and equally in the storage of the liquid and dry phosphorobacterin. All the phages isolated at the time of phagolysis of the culture of B. megaterium var. phosphaticum, strain No. 2, (38 of these were isolated from 1956 up till July 1957), were quite similar in the range of their lytic effect. Of these 38 bacteriophages not one lysed the No. 2 culture, but all were active against the mutants of this culture, namely strains PSA and PSB. Of these 38 phages No. 16 was examined in the greatest detail since we took it as the model in all tests for determining phage-susceptible mutants and in a series of other tests. A brief description of this bacteriophage is presented below. We consider it essential to emphasize that the lysogenicity of the culture does not always appear to be the cause of phagolysis of B. megaterium under industrial conditions.

The phages capable of lysing the B. megaterium culture are widely distributed and we repeatedly discovered various types in the soils. The phages are frequently found in the air at factory sites. There is an especially large number of them during the periods of mass phagolysis. When strain No. 2 was used in the preparation of phosphorobacterin the majority of those phages found in the air were active only against phage-susceptible mutants of this culture (strains PSA and PSB). In individual cases, however, phages were isolated from the air which were capable of lysing strain No. 2 as well.

Therefore the possibility is not to be excluded that in a series of cases the cause of phagolysis could be contamination of an industrial culture by a phage from the air at some stage in the preparation of phosphorobacterin.

Thus at the beginning of 1957 the Kiev phage was isolated from the culture liquid at the time of phagolysis of B. megaterium var. phosphaticum, strain No. 2, at the Kiev Industrial Bacteriological Laboratory. This phage differed sharply in a number of properties, especially with regard to the range of its lytic activity, from the phages isolated at the First State Industrial Bacteriological Laboratory, in particular the model phage 16. The Kiev phage actively lysed the No. 2 culture and all its variants.

In the second half of 1957 there began to be isolated from the culture liquid of the reactors in which phagolysis had been observed phages which lysed the industrial culture strain No. 2 and which were close to the Kiev phage in the range of their lytic activity.

The source of origin of the Kiev phage and of those phages resembling it still has not been definitely established.

It is possible that the industrial culture was contaminated by this phage from without. It is also possible that the Kiev phage could be the virulent variant (mutant) of the symbiotic phage carried by the No. 2 culture. One argument against the latter suggestion is the fact that this phage differs from phage No. 16 in the morphology of its particles (see Figs. 5 and 6), and, according to preliminary data (the data of N. Ya. Solovyova), serologically as well.

Phage No. 16 does not multiply on fish broth and MPB (meat-peptone broth). For the most part it will not lyse susceptible cultures on MPA or its ability to lyse is low. It does lyse actively on the solid or liquid media used at the industrial laboratory, particularly the peptone-corn medium.

When phage No. 16 acts on the cultures susceptible to it, a secondary growth is rapidly formed, consisting of resistant cells.

Phage No. 16 forms plaques which measure from 1 to 2mm in diameter (Fig. 1). The plaques are often surrounded by halos consisting of turbid cells (Fig. 2). The halos appear after 24 hours of growth of the culture and increase in size on the days following, reaching a diameter of 10 cm.

The ability of the Kiev phage to lyse susceptible cultures was high on MPB, MPA, peptone-corn medium and the media used in the industrial laboratory. A secondary growth rarely formed. The plaques of this phage, which measured from 0.5 to 2 mm, were also surrounded by halos (Figs. 3 and 4).

The appearance of halos around the plaques of the No. 16 and Kiev phages indicates that when the B. megaterium was lysed as a result of interaction with these phages, special lytic substances able to diffuse in agar are formed in addition to the particles of phage.

Similar substances in B. megaterium have been described by Murphy (1957), who established that they are enzymes capable of destroying the microbial cell wall. Serologically these substances are distinct from the phage and their formation is connected with phagolysis.

Hence, when the phagolysis of B. megaterium resulted from interaction with the bacteriophages we investigated, in addition to the production of phage particles there occurred the formation of special lytic substances capable in turn of causing the disintegration of the culture, which intensifies the course of lysis. Phages No. 16 and Kiev proved to be quite sensitive to citrates. They did not lyse the susceptible cultures on PCA when there was a 0.5% sodium citrate in the medium; with a concentration of 0.35% and lower, lysis was possible.

Electron microscope study of phages No. 16 and Kiev showed them to be spermatoid in form. The size and form of the heads of both phages were identical, while the tail of the Kiev phage was approximately twice as long as that of phage No. 16 (Figs. 5 and 6).

Comparative data on the range of lytic effect of the phages No. 16 and Kiev presented in Table 2 show that the Kiev phage has a considerably wider range of lytic effect than does phage No. 16.

It is known that to combat phagolysis under industrial conditions successfully, a series of measures absolutely must be carried out. Particularly important is a selection of industrial source cultures which differ

*The authors wish to express their thanks to A. S. Tikhonenko who carried out the electron microscope study of these phages at the Laboratory of Electron Microscopy, Academy of Sciences, USSR.

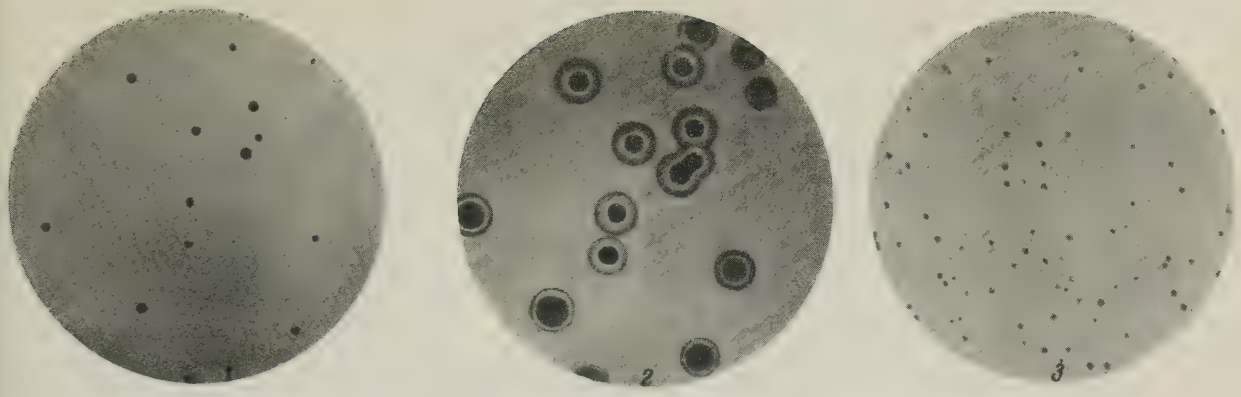
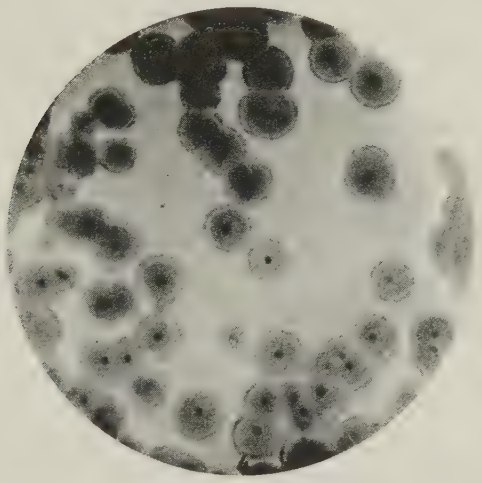


Fig. 1. Plaques of No. 16 phage on a Bacillus megaterium, PSA, culture after 24 hours. 6.6:10
Fig. 2. The same plate after 72 hours. 8:10
Fig. 3. Plaques of the Kiev phage on a culture of Bacillus megaterium, PSA, after 24 hours. 6.6:10



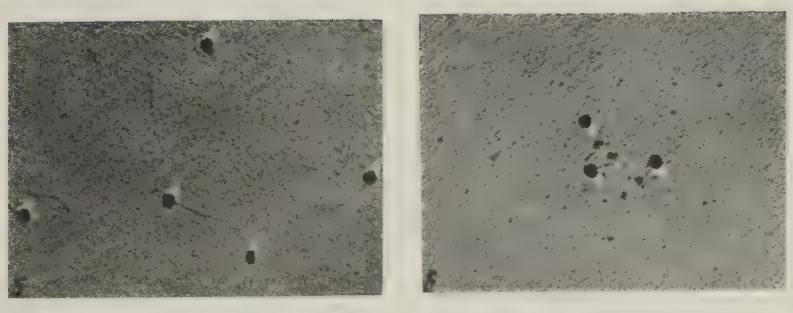


Fig. 4. The same plate after 120 hours. Magnification 8:10 x
Fig. 5. The No. 16 phage. Magnification about 28,000 x
Fig. 6. The Kiev phage. Magnification about 28,000 x

Table 2. The Range of Lytic Activity of Phages No. 16 and Kiev Bacillus megaterium on PCA Medium at 27–28°

Test Cultures	Bacteriophages	
	No. 16	"Kiev"
B. megaterium var. phosph., strain No. 2	—	+
B. megaterium var. phosph., strain PSA	+	+
B. megaterium var. phosph., strain PSB	+	+
B. megaterium, museum strain No. 2	—	—
B. megaterium, museum strain No. 6	—	+
B. megaterium, museum strain No. 7	+	+
B. megaterium, museum strain No. 9	—	—
B. megaterium, museum strain No. 10	—	+
B. megaterium, museum strain No. 11	—	—
B. megaterium, museum strain No. 12	—	—
B. megaterium, museum strain No. 15	—	+
B. megaterium, museum strain No. 18	—	—

NOTE. + indicates susceptibility, — indicates resistance of the culture to the given phage.

Table 3. Comparative Ranges of Lytic Activity of Phages Isolated at the Time of Lysis of the Bacillus megaterium cultures No. 2 and P57

Industrial source strains of B. megaterium	When, where, and by whom the culture was obtained	Phages isolated at time of lysis of B. megaterium cultures			
		No. 2		No. 57	
		16	"Kiev"	87	100
No. 2	1937, Chernozem soil from the preserve of the Scientific Research Institute of Agriculture for the Central Black Earth Belt, by R. A. Menkina	—	+	—	—
PSA	Phage-susceptible variant (A) of culture No. 2	+	+	+	+
P57	1957, garden soil from the city of Pushkin, by A. L. Bychkovskaya	—	—	+	—
R	1957, Ciscaucasian organic Chernozem soil from Rostov Province, by R. A. Menkina	—	+	+	+
Ko	1957, southern Chernozem soil (old tillage) from Kustanay Province, by R. A. Menkina	—	—	+	—
No. 5	1958, chestnut soil from Izmailov Experimental Field of Odessa Province, by I. V. Yaroshevich	—	—	—	—

NOTE. + indicates susceptibility, — indicates resistance to the given phage.

in their susceptibility to various bacteriophages. When cases of phagolysis are discovered the availability of such a collection of cultures makes it possible for the industrial workers to replace one culture with another one which is not susceptible to whatever phages have been isolated. Frequent shifting of industrial cultures has proved to be a very effective means of combatting phagolysis in the manufacture of cheese (Rautenshtein, 1957).

The Institute of Agricultural Microbiology at the All-Union Academy of Agricultural Sciences, which supplies source cultures to the industrial laboratories, is carrying on work with the isolation and study of new cultures of phosphorous bacteria, and at the present time the number of industrial source cultures has been somewhat increased.

Nevertheless, since verification of the suitability of a culture for the manufacture of an active fertilizer takes several years, the number of industrial source cultures is small.

The acceptance into industry of new cultures has brought to light some interesting peculiarities. Thus, when the B. megaterium P57 culture was first being used in the industrial laboratory, the bacteriophages Nos. 87, 100, and others, were isolated at the time of phagolysis of this culture and were found to differ from the phages produced when the No. 2 culture was used.

In Table 3 are presented comparative data on the lytic properties of these phages and of phages No. 16 and Kiev in relation to a series of source cultures.

From the data in Table 3 it can be seen that phagolysis of the various industrial cultures is brought about by a diversity of phages. When lysis occurs in culture P57 or in the No. 2 culture and phages are isolated, some are able to lyse these cultures, while others are not. It is possible to pick out B. megaterium which differ markedly from each other in their susceptibility to the bacteriophages obtained at the time of phagolysis under industrial conditions.

The detailed study of the bacteriophages isolated when phagolysis occurs in the industrial source cultures of B. megaterium and the elucidation of their sources of origin presents itself as a subject for further investigations.

SUMMARY

A study was carried out on the causes of lysis in culture of Bacillus megaterium var. phosphaticum

strain No. 2, when this culture is used in the production of phosphorobacterin under industrial conditions, and of the peculiarities of the bacteriophages causing the lysis. It was established that:

1. The culture of B. megaterium var. phosphaticum, strain No. 2, apparently is one of the noninducible lysogenic strains. It is resistant to the phage it carries, but when cultivated on liquid and solid media it freely produces susceptible mutants. The number of these mutants can be quite considerable. The degree of lysis in the culture when it is cultivated in inoculators and reactors depends on the number of phage-susceptible mutants which have been formed.

2. Phagolysis of a relatively insignificant portion of the cells undoubtedly is a rather frequent occurrence during the production of phosphorobacterin. Hence, the end product (liquid and dry phosphorobacterin) often contains the bacteriophage, sometimes in considerable quantity, which can remain active for a long period of time.

3. Phagolysis could also be caused at various stages in the production of phosphorobacterin by contamination of the culture with a phage from without. This apparently accounts for the fact that in individual cases when phagolysis occurs bacteriophages are also released which are capable of lysing the original inoculum.

4. A more detailed study was made of two bacteriophages obtained when phagolysis occurred in the culture of B. megaterium var. phosphaticum, strain No. 2: phages No. 16 and Kiev which differ in the range of their lytic activity, in virulence, and in the morphology of their particles.

5. The No. 16 and Kiev phages form plaques 0.5 to 2 mm in diameter, which are surrounded by halos of turbid cells. The size of the halos increases in proportion to the growth of the culture, reaching a diameter of 10 cm or more on the third day. The appearance of the halos around the plaques indicates that when phagolysis occurs in the B. megaterium culture as a result of the interaction with these phages, special lytic substances are formed which are diffusable in agar.

6. The No. 16 and Kiev phages are very sensitive to citrates. When 0.5% sodium citrate is present in the medium these phages are unable to lyse cultures susceptible to them.

7. Phagolysis under industrial conditions was also noted in another culture (P57) resistant to the No. 16 and Kiev phages. The phages obtained at that time differed from the No. 16 and Kiev phages in the range of their lytic activity. In addition to the phages not capable of lysing the P57 culture, there also occurred phages which were active against it.

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A STUDY OF THE CONTINUOUS ACETONE BUTYLIC FERMENTATION CAUSED BY CLOSTRIDIUM ACETOBUTYLICUM

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In recent years the continuous fermenting processes which occur in various branches of the fermenting industries have attracted increased interest.

In 1958 an international symposium was held in Prague and this was followed by the All-Union Conference in Moscow which was concerned with the question of cultivating microorganisms in flowing media and with continuous fermentation. The investigations demonstrated that, apart from the advantages of continuous fermentation associated with the acceleration of the process and the depth of fermentation, it was possible to change the microorganisms by adapting the strain to different media including products of fermentation, and thereby to raise the concentration of the fermenting medium. This was demonstrated by Ierusalimskii and Pinaeva on Clostridium acetobutylicum (Pinaeva, 1957; Cont. Cult. of Micr., 1958).

The advantages of continuous fermentation were demonstrated in the alcohol industry by Yarovenko (1958) who showed that its introduction in a number of works raised the productivity of the fermenting sections by 15–19%.

In acetone butyl production, fermentation is carried out in a battery of fermentators which is loaded through an activator with flour mash and with the bacterial culture and is then left for secondary fermentation (Logotkin, 1939). An analysis of this method of fermentation (Yarovenko et al., 1958) has shown that as a result of a too great speed of inflow of mash during the loading of the battery, the number of bacterial cells in the activator diminished to $0.7 \cdot 10^9$ in 1 ml of brew and remained at a low level in all the fermentators of the battery until the termination of loading. The rate of propagation of the microorganisms and the speed of fermentation are approximately three times less than the speed of inflow of mash. This causes a reduction in the effective utilization of the useful volume of the battery.

In the past (Logotkin, 1958), attempts were made to bring about continuous acetone butylic fermentation. The production battery was loaded for 47 hr with maize mash and 35 hr after the commencement of loading a ripe brew with an acetone content of over 7 g/liter was removed from the last (sixth) fermentator. More thorough investigations of the process were not undertaken because of the difficulties involved in carrying them out in production conditions.

PROCEDURE IN A SEMIINDUSTRIAL PLANT

In order to study the continuous fermentation in the Dokshukinsk acetone plant, a relatively large experimental apparatus was constructed on which it was possible to reproduce all the production processes. This apparatus for continuous fermentation is shown in Fig. 1.

The mash vat with a capacity of 5 m^3 (Fig. 1, 12) was filled with an industrial mash which had already been boiled and sterilized. At a temperature of 115° the mash was brought by a rotary pump (13) to the cooler (14) where it was cooled to 37° and then passed to the first fermentator (1) for loading of the battery (1–11). The battery consisted of 11 fermentators with a useful capacity of 3.5 m^3 each and interconnected by pipes 100 mm in diameter. The pipes were equipped with valves designed to switch off the fermentators during sterilization. On these occasions the brew was pumped into the following fermentators (16, 17). The mash after cooling was also fed to the second fermentator of the battery and to the inoculator (15) in which a strain of acetone butylic microorganisms was being cultivated.

The ripe brew during the process of fermentation was diverted to manufacture or was distilled in a fractionating column (18) of the experimental section.

Besides mash, each fermentator was also supplied with sterile air,* steam, and water. A gas line led off from each fermentator through a foam trap (19) into the atmosphere. All the fermentators were equipped with irrigation rings for cooling after sterilization and, when necessary, during fermentation. The upper bottoms of the fermenting vats were equipped with hatches which could be hermetically sealed. Fermentation was carried out under a pressure of 0.2–0.3 atm to prevent infection from without.

The apparatuses were equipped with manometers, thermometers, water gauges, and gauge cocks. All the closed sections of the production lines had steam plugs to preserve the sterile conditions.

*Sterile air in acetone butylic production is used for maintaining the pressure in the fermentators during the period of low evolution of gas and for cooling them after sterilization.

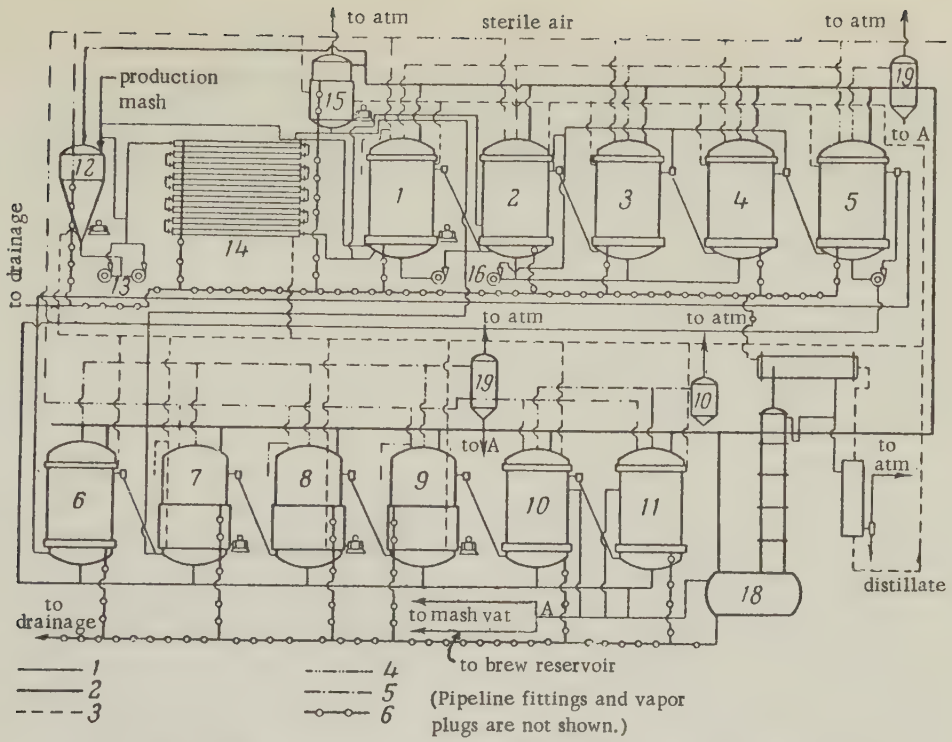

Fig. 1. Schematic diagram of the experimental apparatus for continuous fermentation. Conventional signs of the pipelines. 1) Production; 2) steam; 3) water; 4) air; 5) gas; 6) draining.

The working process of the fermenting apparatus was as follows. The battery was loaded with a sterile mash at a rate of 0.6–1.2 m^3/hr through the first fermentator, which contained an active strain. After loading of the whole battery, which took from 35 to 60 hr, the ripe brew was removed continuously from the last fermentator.

After definite periods of time (in our tests from 96 to 120 hr), all the fermentators of the battery were successively sterilized. Before the commencement of sterilization, the contents of the first fermentator were pumped into the second fermentator; at the same time the inflow of mash into this part of the battery was interrupted. After discharge and washing, the first fermentator was sterilized simultaneously with the vat, the cooler, and the loading line. At the same time, the brew from the second fermentator was pumped into the third and thus a prophylactic sterilization of the vats of the battery was carried out.

During the sterilization of the second fermentator, the inflow of mash into the first fermentator was resumed—on a freshly prepared strain of acetone butylic organisms. From this moment the next work cycle of the fermentating battery began.

From each fermentator of the battery, every 4–8 hr, brew specimens were taken and were then used for determining acidity, in milliliters 0.1 N† per 10 ml, the total sugar after an acid hydrolysis (Nakhmanovich and Shcheblykin, 1958), and the number of bacterial cells in 1 ml.

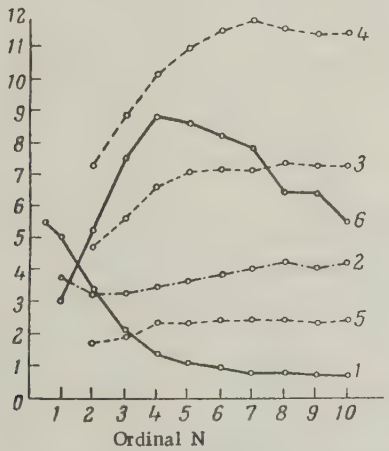

Fig. 2. Dynamics of continuous acetone butylic fermentation. 1) Total sugar (%); 2) Titratable acidity (ml 0.1 N in 10 ml); 3) Acetone (g/liter); 4) Butanol (g/liter); 5) Ethanol (g/liter); 6) Number of microorganisms ($n \cdot 10^9$ in 1 ml).

EXPERIMENTAL RESULTS

The battery was continuously loaded with mash through the first fermentator, which contained an active strain of organisms, and the ripe brew from the tenth fermentator was continuously used for distillation.

The investigations have demonstrated that in the process of continuous fermentation, in each fermentator of the battery constant conditions were established which correspond to a specific stage of acetone

†As in original-Publisher.

Table 1. Characteristics of the Different Cycles of Continuous Fermentation

Cycle No.	Speed of battery loading (m^3/hr)	Rotation of each fermentator (in hr)	Duration of loading (hrs)	Am't mash passed through battery	Inoculator		No. fermentators necessary for termination of fermentation	Ripe brew			
					maximum acidity	hour of discontinuity		test battery		production indices	
								acetone, g/l	residual sugar, %	acetone, g/l	residual sugar, %
1	First 48 hr. 1.3 then, 0.9	7.5 5.4	91	101	5.2	4	9	6.5 6.9	1.02 0.71	6.9	0.71
2	0.65	4.5	114	74	5.0	16	8	6.8	0.95	6.7	0.73
3	0.65	4.5	107	70	5.8	17	7	7.1	0.6	7.0	0.70
4	0.65	4.5	78	50	4.9	21	7	6.75	0.94	7.1	0.70
5	0.62	4.3	73	47	5.0	—	8	6.8	0.65	7.0	0.71
6	0.59	4.2	72	40	6.4	—	8	6.9	0.60	7.0	0.79

Table 2. Fermentation Conditions in Test Batteries

Battery No.	Speed of loading (m^3/hr)	Rotation of each fermentator (hrs)	Starchiness of the mash (%)	Duration of loading (hrs)	Amount of mash passed through the battery (m^3)	Conditions for fermentation termination		Speed constants of fermentation	Coefficient of overfilling*
						no. of fermentator	duration (hrs)		
1	0.55	3.80	5.00	190	108	5	31.8	0.1044	1.0
2	0.68	4.65	—	75	52	6	31.0	0.0969	1.0
3	0.87	5.70	5.56	170	147	7	29.5	0.1163	1.05
4	1.00	5.35	5.00	90	87.5	8	36.4	0.0749	1.3
5	1.17	6.25	4.75	101	110	9	35.0	0.0816	1.3
6	1.18	6.30	4.90	136	150	9	34.6	0.0790	1.3
7	0.70	4.40		243	171	5	27.2		1.06
8	0.64	4.10		271	175.5	5	30.0		1.05

*At loading speeds of over 1.0 m^3/hr, a resistance was built up in the pipes and the fermentation vats became overfilled. The coefficient of overfilling of the loaded battery reached 1.3; this was taken into account in calculating the revolutions and speed of fermentation.

butylic fermentation after the break of the acidity curve. This can be clearly seen in the curve plotted from the average figures of the analysis for the whole period of fermentation (Fig. 2).

At the beginning, fermentation was carried out without prophylactic sterilization of the apparatus and pipelines up to the moment of infection of the brew; this made it possible to determine the duration of the cycle. The brew became infected 124–168 hr after continuous loading; until then the fermentation indicators were normal.

In further tests continuous fermentation was carried out with prophylactic sterilization every 96–120 hr. In this way it was possible to have continuous fermentation over a period of 672 hr (with 6 sterilization cycles) without the brew becoming infected.

The mash used for fermentation was industrially prepared from flour which does not ferment easily and which was ground from intergrown grains. The mash was boiled at 150–153°, maintained at that temperature for 23 minutes, and then cooled.

The results of the analyses of individual cycles are presented in Table 1.

The study of continuous fermentation in a number of other test batteries was directed to the elucidation of the influence of rate of loading on the process of fermentation.

At the same time other factors were also studied. Beginning with the third battery, the brew in the first fermentator was subjected to periodic mixing. Batteries were loaded with mash at various speeds from 0.55 to 1.2 m^3/hr. The principal indicators of fermentation of eight batteries at different speeds of loading are presented in Tables 2 and 3.

The loading of battery No. 2 was discontinued at the seventy-sixth hour, as the turbine had stopped. Batteries No. 4 and 6 were loaded for 90 and 136 hours respectively in accordance with the plan of investigation. In these batteries the brew did not become infected.

Brew in batteries Nos. 1 and 3 began to get sour after 185 and 170 hrs of loading, as a result of infection by wild lactic acid bacteria. Infection occurred much earlier in battery No. 5 where by the sixty-fifth hour of fermentation lactic acid rods were found in each field of vision of brew smears taken from fermentators 6, 7, and 8. Acidity, however, during this period remained within the usual limits. The formation of solvents was also normal.

These observations demonstrate that in conditions of continuous fermentation a lactic acid infection which gets into the battery affects the original strain much more slowly than in conditions of periodic and battery methods of fermentation. Thus, even when lactic acid

Table 3. Fermentation Indices of Test Batteries

Battery no.	Average number of bacterial cells in fermentators ($n \cdot 10^9$)		Analysis of ripe brew							
			Test batteries						Production indices	
			Solvents (g per liter)				Residual sugar (%)	Acidity ml 0.1 N per 10 ml		
	No. 1	No. 3-4 maximum	Acetone	Butanol	Ethanol	Total			Acetone (g/l)	Residual sugar (%)
1	3.0	4.2	7.1	—	—	—	0.50	4.3	6.9	0.66
2	2.1	—	7.4	—	—	—	0.66	4.2	7.0	0.67
3	3.0	8.8	7.2	11.5	2.4	21.1	0.72	4.0	7.1	0.73
4	—	—	6.94	11.32	2.62	20.88	0.64	3.9	7.0	0.74
5	1.76	7.8	6.75	11.03	2.18	19.96	0.60	4.1	6.95	0.63
6	—	—	6.08	11.27	1.97	19.32	0.72	4.1	—	—
7	—	—	7.03	—	—	—	0.62	3.8	6.6	0.79
8	—	—	7.0	—	—	—	0.63	3.9	7.0	0.71

rods were clearly present it was possible to load the battery for a period of 30 hr during normal fermentation. Infection during fermentation infiltrates mainly through the many stuffing boxes of the pumps, mixers, and gate valves. In order to prevent infection from taking hold we designed special stuffing boxes with antiseptic oil in which copper sulfate was dispersed. Such an oil, without losing its lubricating properties, acts as a strong antiseptic. Once the whole assembly of the fermenting battery had been equipped with these stuffing boxes, it was possible to increase considerably the duration of fermentation without sterilizing the apparatus and communications. The batteries were continuously loaded for 240-264 hr (batteries Nos. 7 and 8, Tables 2 and 3).

After 240 hrs of continuous loading the strain generally became weaker, the bacterial cells increased in size, the acidity of the medium rose to 5-6 ml 0.1 N‡ per 10 ml of brew, although there was no extraneous microflora.

Continuous fermentation can be brought about at different speeds of inflow and correspondingly with different turnover of the fermentators. By turnover, we mean the amount of mash or brew which passes through a given fermentator in the course of 24 hr, and which is expressed in units of useful capacity of the given apparatus. On increasing the inflow, the minimum number of fermentators necessary in a battery increases. Thus, at a loading speed of 0.55 m^3/hr five fermentators were sufficient, but at a speed of nearly 1.2 m^3/hr, nine were required.

Table 3 presents the average production indices of fermentation (acetone content and residual sugar in the brew) for the same periods of time that fermentation was going on in the test batteries, i.e., with the same mash and in the presence of the same bacterial spores. A comparison of these results with the corresponding indices of continuous fermentation shows that in the latter case the fermenting of the mash was better than in industrial conditions. A certain decrease in the acetone content in the fifth battery was associated with the infection of the brew as already mentioned above.

Fermentation lasted from 27.2 to 36.4 hours.

As we have already shown (Yarovenko et al., 1958) the process of acetone butylic fermentation is governed by the law of monomolecular reaction. The speed constants of fermentation make it possible to compare the processes of fermentation which occur in different conditions or during the fermentation of various raw materials.

The character of the change in the number of bacterial cells in conditions of continuous periodic and semicontinuous methods of fermentation varies. In periodic fermentation, the number of bacterial cells reached its maximum—nearly $4 \cdot 10^9$ in 1 ml—between the fifteenth and twentieth hour of fermentation, remained within these limits until approximately the thirtieth hour of fermentation, and then decreased (lysis), reaching $1 \cdot 10^9$ by the fiftieth hour.

In semicontinuous fermentation at the moment when the activator ceased to function it contained about $3.5 \cdot 10^9$ bacterial cells in 1 ml of brew. With the commencement of loading, the number of organisms in the activator decreased to $0.7 \cdot 10^9$ in 1 ml. Only after the termination of loading did the number of bacterial cells begin to increase, reaching its maximum between the thirtieth and fortieth hours of fermentation, from $5.3 \cdot 10^9$ in the activator to $7.8 \cdot 10^9$ in the last fermentator of the battery. Thereafter, the number of bacterial cells decreased sharply to $2.5-4.0 \cdot 10^9$ in 1 ml by the fortieth to forty-sixth hour of fermentation.

In continuous fermentation, the number of organisms rapidly increased at the beginning of loading, and remained at a high level in all the fermentators of the battery throughout the whole period of loading. The number of organisms in the first fermentator was $2.5-3.4 \cdot 10^9$, whereas in the other fermentation vats, it reached $11-12 \cdot 10^9$ in 1 ml, while the average values in individual fermentators were $8.6-8.8 \cdot 10^9$.

During the process of continuous fermentation, a strain was removed from the battery for spore formation in view of the possible changes which the organisms might undergo in these conditions.

SUMMARY

1. The study of the process of continuous acetone butylic fermentation was carried out on an experimental semiindustrial plant with a fermentation battery

‡As in original-Publisher.

consisting of 11 fermentators each with a useful capacity of 3.5 m^3.

When the battery was loaded with flour mash at a rate of 0.6–1.2 m^3/hr, and with the continuous delivery of brew, fermentation proceeded normally without sterilization for a period of 144–264 hr. Thereafter, the fermenting mass became infected with lactobacilli or the acetone butylic strain became attenuated, this usually happening after 240 hr of continuous loading.

2. The following were investigated: the character of the fermented sugar, the formation of solvents, the accumulation of bacterial cells, and the changes in acidity and pH. The fermented media were found to contain over 7 g/liter of acetone and nearly 0.65% of residual sugars.

In each fermentator of the battery, constant conditions were established which corresponded to a definite stage of acetone butylic fermentation.

3. Continuous fermentation with prophylactic sterilization of the equipment and piping was carried out in the course of 672 hrs without any infection of the brew. During this period 6 sterilization cycles wer carried out, each of which was started on a new strai of acetone butylic organisms. All fermentation indice were normal.

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CAUSATIVE AGENTS OF THE BIOLOGICAL DECREASE OF ACIDITY IN APPLE JUICES AND CIDER

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For a long time the observed biological decrease of acidity in grape and fruit-berry wines were ascribed only to the activity of microorganisms (Pasteur, 1861; Muller-Thurgau and Osterwalder, 1913; Korablev, 1931; Sichert, 1937; Schanderl et al., 1939).

Microorganisms which bring about a decrease in the acidity of wines include lactobacilli of the Bacterium gracile type and cocci—Micrococcus variococcus, M. acidovorax, M. malolacticus which break down malic acids into lactic acid and carbon dioxide.

Theoretically, 1 g of malic acid yields 0.67 g of lactic acid and 0.33 g of carbon dioxide.

The bacterial decrease of acidity occurs slowly at raised temperature and, as a rule, after vigorous fermentation, whereas at lower temperatures, it can last for several months.

The development of acidity-decreasing microorganisms is completely inhibited by 75–100 mg/liter of SO_2.

A pH value below 2.9 is critical for the biological reduction of acidity.

Chalenko (1941), from a study of the acidity reduction in fruit wines (apple, cherry, plum) in the Voronezh, Ostrogozh, Kursk, and Annensk wine-making enterprises, established that the cause of the acidity reduction was not bacteria, but the formation of yeast which had not until then ever been mentioned either in Soviet or foreign literature as an acid reducer.

The reduction in acidity of apple wines occurred in the main at the moment of vigorous fermentation and came to an end between the 96th and 168th hours of fermentation. The malic acid, moreover, disintegrated almost completely into carbonic acid and water. The isolated microorganism was ascribed, on the basis of morphological-physiological studies, to a new species of the genus Schizosaccharomyces and named S. acidodevoratus. However, a more accurate name for the species is Schizosaccharomyces acidodevorax (Chalenko, 1946) and this is the one to be used.

In contrast to the acidity-reducing microorganisms, acidity-reducing dividing yeasts can tolerate large concentrations of sulfurous anhydride (up to 800–1000 mg/liter).

Kudryavtsev (1954) from his study of the specie composition of the genus Schizosaccharomyces recorded only two species:

1. S. Pombe, found in tropical and subtropical countries, develops in sugary starchy substrates (clear beer, cane molasses, rice mash, and so forth).

2. S. acidodevorax (acidodevoratus) are distributed in the more northern countries of the globe. They have been found in the Soviet Union and Switzerland in fruit and berry juices and wines. Besides fermenting sugars they have the ability to decompose malic acid. The industrial production of cider in the Soviet Union began about 1949.

As a result of the employment of new technology in the production of cider it was observed that during the reprocessing of fruit, and in the course of fermentation of apple juices as well as during storage of fermented ciders, there were many instances of acidity reduction in the wine-making enterprises of Crimea, northern Caucasus, northern Osetia, Transvolga, Belorussia, and other districts.

Apple juice or cider which had been subjected to acidity reduction became brownish-black in color and turned into an acid-free, flat, watery product, unsuitable for use.

To study the spontaneous microflora of apple juices subjected to acidity reduction, a microbiological examination of 47 specimens of juices from different districts was carried out.

The microflora of the juice consisted in the main of yeast cells of the Saccharomyces vini (Saccharomyce ellipsoideus) type, Hanseniaspora apiculata, Saccharomycodes, Schizosaccharomyces. Occasionally we encountered single organisms of the lactobacilli type, as, for example, in the fermented apple juice of the northern Osetia combine, of the Belorussia wine trust, of the Armavir wine factory, and others (Fig. 1).

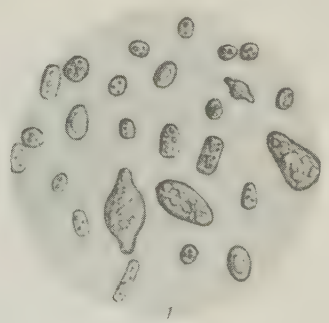

Fig. 1. Spontaneous microflora in a sample of acid-reduced apple juice from the Maikop district.

Subsequently, organisms capable of bringing about a reduction in the acidity of apple juices were isolated and studied. The investigations showed that only those isolated strains which belonged to the genus Schizosaccharomyces possessed the ability to reduce acidity. The other groups of yeast did not have these properties.

Fifty-three strains of pure cultures of dividing yeasts were isolated, but, as they were all identical, the following were selected for further study:

1. Schizosaccharomyces No. 9 northern Osetia.
2. No. 6 Simferopol'
3. No. 5 Simferopol'
4. No. 12 Feodosiisk
5. No. 3 Starokrym
6. No. 45 Maikop
7. No. 44 Armavir
8. No. 40 Krasnodar
9. Belorussian
10. No. 1 Kazan
11. No. 4 Stalingrad
12. Moscow

BRIEF CHARACTERISTICS OF ISOLATED STRAINS

In apple juice (acidity 8.5 g/liter, saccharinity 9.7% and pH 3.3), yeast grows by the transverse division of cells. The principal cell shapes were cylindrical, short-cylindrical and rounded. Less frequent were the elongated-cylindrical shapes (Fig. 2).

The dimensions of the cells in a four-day culture were 6.6–23.4 × 3.3–6.6μ.

Strains No. 9 (north Osetia), No. 1 (Kazan), and Belorussia had large rounded and oval cells. Their sizes were 3.3–8.25 × 3.3–5.0μ.

Strain No. 6 Simferopol' had narrow rodlike and elongated-cylindrical cells. Their sizes were 6.6–23.1 × 2.31–3.96μ.

The cells were distributed singly or in pairs. Practically all the cells had a granular plasma and did not contain glycogen, as on staining with an iodine solution, they turned a light yellow color. There was likewise very little fat.

In three-week cultures, an accumulation of fat in the form of several drops was observed in the cells.

In month-old cultures in the majority of the investigated strains, the cells were smaller, short-cylindrical and rounded in shape, and had a more granular plasma. The dimensions of the cells were 3.3–16.3 × × 3.3–6.6μ.

The exception was strain No. 6 Simferopol', the cells of which underwent no changes either in size or shape.

The discovery of the acidity-reducing dividing yeasts has a great practical significance in the microbiological control of production.

After a period of 15–40 hr most of the strains—with the exception of No. 6 Simferopol'—produced spores on gypsum blocks.

Spore formation was preceded by the copulation of pairs of cells. The spores were oval in shape, 4 spores to an envelope. The spores were distributed along the cell or crosswise; the sizes were 3.0–3.3 × 2.6–3.0μ.

On a yeast agar medium with 0.5 g/liter of malic acid and 1% glucose, all the strains formed spores within 38–54 hr. Each sack had 2–4 spores which were arranged along the length of the cell or crosswise.

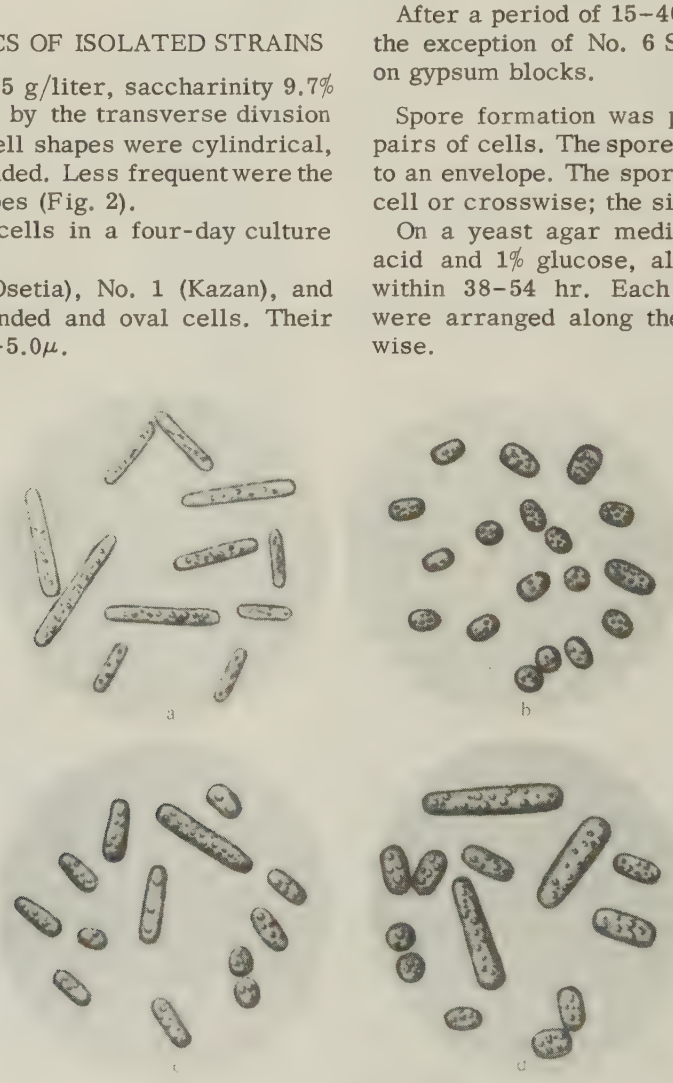

Fig. 2. Shape of pure yeast cultures Schizosaccharomyces in apple juice (a 96-hour culture). a) No. 6 Simferopol'; b) Belorussian; c) No. 3 Starokrym; d) No. 12 Feodosiisk.

Spore-forming cells sometimes acquired diverse shapes: elongated, angular, horseshoe, or zig-zag shapes.

Spores of oval shape were of the same size as on the gypsum blocks (Fig. 3).

Fig. 3. Spore formation by Schizosacchromyces No. 3 Starokrym.

GROWTH OF YEAST CELLS ON LIQUID AND SOLID MEDIA

In apple juice at 25°, fermentation occurred within 48–72 hr and lasted from 168–240 hr, resulting in a further lightening of the juice color. Neither a film nor a ring formed on the surface of the juice in the tubes.

The color of the yeast precipitate ranged from a greyish-yellow to a light brown in most of the cultures. The yeast precipitate was dustlike. As a result of the decrease in acidity the juice changed color from light yellow to brown with a blackish tint.

Only strain No. 6 Simferopol' had no effect on the color of the juice.

In apple juice with agar (linear sowing) a yellowish-white, greyish-white, or greyish-yellow, dim film with an even surface appeared on the surface.

On sowing by inoculation (apple juice + agar), in most cases growth occurred in the upper part of the puncture and was continuous with the stab in the medium. Certain strains had a stab line resembling a knobby nail (No. 5 Simferopol', No. 4 Krasnodar, and Moscow strain).

When sowings were made by inoculation in apple juice with gelatine, growth likewise occurred in the upper part of the puncture, and caused the gelatine to dissolve and the medium to darken.

Only strain No. 6 Simferopol' had a growth continuous with the test in the medium; moreover, it did not dissolve gelatine nor change the color of the medium.

ASSIMILATION OF CARBON BY DIVIDING YEASTS

The study was carried out on a Rider medium. Glucose, fructose, galactose, saccharose, maltose, and lactose were investigated. Organic acids: tartaric, citric, malic, lactic, succinic, acetic, potassium tartrate, and ethyl alcohol and mannitol. Maltose proved to be the best source of carbon supply and it caused intensive growth of all the tested strains of dividing yeasts.

Glucose also was assimilated well by all strains, with the exception of strain No. 6 Simferopol', which showed only slight growth.

Fructose and saccharose were assimilated less well.

The following were not assimilated: galactose, lactose, the organic acids—tartaric, citric, malic, lactic, succinic, and acetic, potassium tartrate—and the mannitol and ethanol. Nevertheless, in a liquid nutrient media with glucose and malic acid, the latter were assimilated by all the tested cultures.

ASSIMILATION OF NITROGEN

The study was carried out in a Lander medium with the addition of 1% experimental nitrogen source: ammonium sulfate, potassium nitrate, calcium nitrate, peptone, asparagine, glycocoll, and urea.

The best source of nitrogen for the study of dividing yeasts was peptone, which caused intensive growth. The other sources were not assimilated by the dividing yeasts.

A microscopic examination of the yeast precipitate (from peptone) showed that all the strains had the usual cylindrical cell shape except for strain No. 6 Simferopol', most of whose cells were in the form of long, narrow rods, as in the bacteria. The sizes were 6.6–21.45 × 1.65 μ.

FERMENTATION OF SUGARS

The experimental dividing yeasts fermented glucose, fructose, saccharose, maltose, 1/3 raffinose, mannose, and dextrin of malt wort. They did not ferment lactose and galactose.

EFFECT OF TEMPERATURE ON FERMENTATION

The most intense fermentation was observed at 28–30°.

Most of the pure cultures caused intensive fermentation of the juice over a period of 22–27 hr. At 25–26° fermentation was less intense and occurred after 66–90 hr. Slight fermentation was observed at 15–16°. At 6–10° no fermentation was recorded over a period of 360 hr.

All this shows that strains of dividing yeasts from different districts of the Soviet Union developed better at a higher temperature than tartaric yeasts. As tartaric yeasts (S. vini) are more stable at low temperature than the investigated dividing yeasts, it is therefore better to ferment apple juices at lower temperatures (13–15°) in order to overcome acidity reduction.

EFFECT OF pH ON THE PROCESS OF ACIDITY REDUCTION

At pH values of 2.6–4, hydrogen ions had a varying effect on the acidity reduction of apple juice. The greatest reduction in acidity was recorded when the pH was 4.0 (during alkalization). The process of reduction was severe and by the ninety-sixth hour the malic acid had completely disintegrated. In apple juice with a pH of 2.6–3.0 acidified with malic acid the process of acidity

reduction was likewise vigorous; by the forty-eighth hour the acid content had decreased by 70–75%; by the seventy-second hour, by 80–87%.

FERMENTING PROPERTIES OF DIVIDING YEASTS

Fermenting properties were studied on sulfitized apple juice, which, after desulfitization by heating, had the following composition.

Titratable acidity –9.2 g/liter of malic acid, sugar–8.48%, specific weight–1.030, pH–2.9. The total amount of sulfur dioxide was 92.1 mg/liter of which 14 mg/liter was free sulfur dioxide.

Apart from the isolated yeast strains, we also tested previously isolated acidity-reducing yeasts S. acidodevorax as well as tartaric yeasts S. vini (with the Malic strain No. 7) as control.

The juice was poured into retorts with a capacity of 400 ml, 270 ml into each retort and twice sterilized by circulating steam for one hour with an interval of 24 hr. Of the cultures under investigation, 0.5% were then introduced into the retorts, which were sealed with fermentative stoppers with sulfuric acid and left to ferment at 23–27° (table).

From the table it will be observed that pure cultures of dividing yeasts fermented juice for a period of 408–672 hr. Particularly high fermenting properties were manifested by strains No. 44 Armavir and No. 3 Starokrym which fermented juice for a period of 408–432 hrs, evolving 11.4 g of carbon dioxide.

The titratable acidity, excluding the volatile acids of the fermented juice, decreased in most retorts by 99.46–85.8%. The greatest decrease in acidity was produced by No. 3 Starokrym, No. 40 Krasnodar, No. 12 Feodosiiskii, and No. 45 Maikop.

A lesser decrease in the acidity of apple juice was caused by strains S. acidodevorax and Moscow Schizosaccharomyces–by 78.8–65.8%. Strain No. 6 Simferopol' produced the smallest reduction in the acidity of apple juices of all–by 22.0% of the initial acidity.

Moreover, the strains which we isolated and studied were vigorous excitants of the alcoholic fermentation of sugars. The amount of alcohol formed in cider varied from 4.9 to 5.2% (volumetric). The content of volatile acids in ciders ranged from 0.47 to 0.54 g/liter in relation to acetic acid. If we deduct from the titratable acidity the amount of volatile acids, then the decrease of acidity in cider will be greater still.

As a result of the decrease of titratable acidity in cider, the actual acidity also decreased and the pH increased in most retorts from 2.9 to 3.6–4.2.

A study of the dynamics of acidity reduction showed that decomposition of malic acid is so rapid that by the ninety-sixth hour, in 17 retorts the titratable acidity decreased from 9.2 to 1.8–2.0 g/liter. Subsequently the decrease in acidity of apple juice continued, but not as vigorously. Thus, by the 240th hour, the acidity decreased to 1.0–1.5 g/liter.

The process of acidity reduction is slower in apple juice fermented by S. acidodevorax. This demonstrates that pure cultures of dividing yeasts isolated in southern districts are stronger acidity reducers.

In the wort fermented on a pure culture, Malic No. (S. vini), the acidity remained unchanged.

Acidity reduction in apple juices and cider differs from bacterial acidity-reduction in that it takes place mainly during the period of vigorous fermentation and is characterized by rapid disintegration of malic acid without the formation of lactic acid.

SUMMARY

1. New strains of dividing yeasts have been isolated which, because of their morphological-physiological characteristics, we have ascribed to Schizosaccharomyces acidodevorax.

2. The isolated strains have the ability to ferment sugars with the simultaneous fermentation of alcohol and to oxidize malic acid to CO_2 and water, thereby causing a decrease in the acidity of apple juices and cider.

The most active acidity-reducers are the strains isolated in the southern districts of the Soviet Union: No. 3 Starokrym, No. 9 North Osetia, No. 40 Krasnodar, No. 4 Maikop. Compared with the strain S. acid

Strains Schizosaccharomyces	Duration of fermentation (in days)	Amount of carbon dioxide evolved in g per 270 ml	Result of analysis: Titratable acidity, g/liter malic acid	Nonvolatile acids, g/liter malic acid	Volatile acids, g/liter acetic acid	Alcohol, % volume	Sugar, %	pH
No. 9 North Osetia	24	11.23	1.5	0.9	0.54	5.1	0.02	3.7
No. 6 Simferopol'	24	10.3	7.7	7.18	0.47	4.85	0.3	3.0
No. 5 "	24	11.39	1.5	0.9	0.54	5.15	0.0	3.7
No. 3 Starokrym	18	11.4	0.6	0.05	0.5	5.2	0.0	4.3
No. 12 Feodosiiski	25	11.31	1.4	0.5	0.54	5.1	0.02	3.7
No. 40 Krasnodar	25	11.52	1.0	0.4	0.54	5.2	0.0	4.2
No. 44 Armavir	27	11.41	1.9	1.3	0.54	5.1	0.2	3.6
No. 45 Maikop	24	11.34	1.3	0.67	0.57	5.2	0.0	3.7
Moscow	28	11.25	3.9	3.15	0.68	5.1	0.0	3.0
Sudak	24	11.22	2.1	1.4	0.6	5.1	0.02	3.7
No. 1 Kazan*	22	11.3	0.2	—	—	6.6	0.17	5.25
No. 4 Stalingrad*	23	11.4	0.2	—	—	6.65	0.14	4.65
Acidodevorax	30	9.86	2.7	1.95	0.68	4.5	0.6	3.0
Saccharomyces vini	14	10.29	9.0	3.4	0.54	4.65	0.7	2.9

* Were tested on a different juice composition.

odevorax, loss of acid amounts to 80.5–97.8% of the original level.

3. The hydrogen-ion concentration has a varying effect on the process of biological acidity reduction caused by dividing yeasts. It is particularly pronounced at pH 4.0, when malic acid is completely disintegrated.

4. The most suitable temperature for development and fermentation is 28–30°. At 15–16° fermentation proceeds slowly, whereas at 4–10° it does not occur at all over a period of 260 hr. This fact must be borne in mind when combatting acidity-reduction by fermenting apple juices at lower temperatures (13–15°), as at these temperatures cold-resistant strains of Sacchar vini (Sacchar ellipsoidens) develop and ferment normally.

5. The isolated strain No. 6 Simferopol' differs greatly from S. acidodevorax by the rod-like shape of its cells and by the fact that it causes only a slight decrease in the acidity of apple juices.

6. The decrease in the acidity of apple juices and cider observed in a number of wine-making enterprises of different districts of the Soviet Union (Krasnodar, Crimea, Pyatigorsk, Saratov, Stalingrad, Belorussian SSR, and others) differs greatly from bacterial acidity-reduction and is caused by different strains of S. acidodevorax.

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COMPARATIVE ACTIVITY OF YEAST STRAINS "KRASNODARSKAYA" AND "KRASNODAR SALTY YEASTS" (KSY) IN THE PRODUCTION OF LIQUID YEASTS

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For several years in the bakeries of the Krasnodar region liquid yeasts containing 0.8% of salt have been used. These yeasts, when diluted to the same extent as ordinary unsalted yeasts, produced bread of good quality (Donchenko and Ponpa, 1957; Donchenko and Kuz'menko, 1958). It was quite natural that prolonged cultivation of yeasts in salt-containing mash should cause the yeast cells to develop new properties. The cells became more osmotic, salt-resistant, and adapted to new conditions of the medium. To distinguish them from the original yeast strain "Krasnodarskaya" they were called "Krasnodar salty yeasts" (KSY).

As a test in some bakeries, salt was added to liquid yeasts prepared from the usual yeast strains but the results were negative. In the literature there are many references to the decrease of the rate of multiplication and to the fermenting activity of yeast microorganisms in the presence of cooking salt. This was established by many of our experiments (Berzina et al., 1957; Roiter et al., 1957, 1959a). White (1957) showed that salt had an inhibiting effect even on osmotic yeasts.

In order to elucidate the reasons for the positive results obtained in work with yeasts "KSY" in spite of the inhibiting action of salt on yeast cells, we investigated the activity of the yeast strains "KSY" and "Krasnodarskaya" during their cultivation both with and without salt.

Yeasts S. cerevisiae of the "Krasnodarskaya" and "KSY" strains were isolated in identical conditions from pure cultures and cultivated concurrently in unsalted and salted media. The salted medium contained 0.8% salt and was prepared in the same way as in industry from a brew of wheat flour (Type II), the proportions of flour to water being in the ratio of 1:3, and leavened by lactate bacteria Thermobacterium cereale strain "E-1" (Roiter et al., 1959b), and mixed with water to 90% moisture. Equal amounts of the same nutrient medium were used for all other tests. Salt was added in the form of a saturated solution. To the control, equivalent amounts of water were added. In all, 24 passages were made, of which 18 were transplanted every 12 hr, leaving a remainder of 25% of ripe yeasts; 6 passages were transplanted every 4 hr, leaving a remainder of 50%. Every 2-3 passages, before transplantation, the acidity, content of yeast cells in 1 ml of liquid yeasts, and the raising capacity were checked by the "ball" method. The results are presented in Tables 1, 2, and 3.

The results in Table 1 demonstrate that in all the tests, the addition of salt to liquid yeasts reduced their acidity on an average by 0.75°. This happened to the same degree in both yeast strains and is explained by the inhibiting effect of salt on the activity of bacterial microflora of liquid yeasts.

Equally noteworthy is the fact that in all the tests, both with and without salt, the acidity of liquid yeasts of the strain "KSY" was higher (on an average by 5°) than in liquid yeasts of the "Krasnodarskaya" strain. As the strains were cultivated in one and the same nutrient medium in identical conditions this indicates a greater formation of acids by the "KSY" yeast cells.

The multiplication of yeast cells as shown in Table 2 decreased when salt was present in liquid yeasts. This was observed in both yeast strains although the inhibiting action of salt on "KSY" yeasts was somewhat slighter. The addition of salt caused a decrease of 13.5% in the number of cells of the "Krasnodarskaya" strain and a 10.5% decrease in the "KSY" strain. Cells of the "KSY" strain were smaller than those of the "Krasnodarskaya" strain: $3-4 \times 5-6\ \mu$ and $4-5 \times 7-8\ \mu$ respectively. On a wort-agar, the "KSY" cells showed only a slight growth, whereas the "Krasnodarskaya" strain grew profusely.

A comparison of the great colonies of pure cultures of the yeast strains under investigation, cultivated by K. A. Kirova in identical conditions on wort-gelatine with 1% salt and without salt, showed that the addition of salt to the medium produced a sharp decrease in the size of the colonies (Fig.).

The addition of salt also had a slight effect on the raising capacity of both strains. However, the inhibiting action of salt was less severe on "KSY" yeasts (Table 3).

In order to provide a double check of the effect of salt on the fermenting properties of yeasts, we also determined the loss in weight of the nutrient medium as a result of the liberation of carbon dioxide during fermentative by liquid yeasts. Ninety grams of flour-leavened brew and 70 ml of water were added to 50 ml of liquid yeasts in retorts with sulfuric acid seals. To

Table 1. Acidity of Liquid Yeasts in Degrees

Passage No.	Strain			
	Krasnodarskaya		"KSY"	
	Without salt	With salt	Without salt	With salt
4	13.0	12.2	13.4	12.8
6	12.6	12.2	13.4	12.6
8	13.2	12.6	14.2	13.4
10	12.8	12.0	13.2	12.0
12	12.2	11.2	12.8	11.4
14	12.4	12.0	13.6	13.0
15	12.6	11.4	12.8	11.8
18	11.8	10.8	11.8	11.2
20	11.2	10.8	11.8	11.2
24	10.1	9.7	10.2	9.8
Average	12.2	11.5	12.7	11.9
Difference	0.7		0.8	

Table 2. Number of Yeast Cells in 1 ml of Liquid Yeasts in Millions

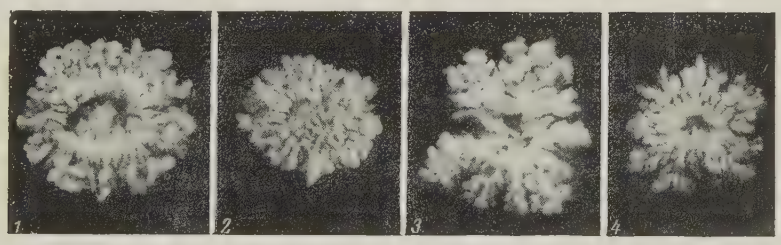
Passage No.	Strain			
	Krasnodarskaya		"KSY"	
	Without salt	With salt	Without salt	With salt
4	77	73	73	64
6	64	62	73	69
10	74	69	72	62
12	66	62	60	56
14	83	62	54	48
18	80	52	68	56
20	76	59	64	57
24	76	75	70	65
Average	74	64	67	60

Table 3. Raising Capacity of Liquid Yeasts Measured by the "Ball" Method in min.

Passage no.	Strain			
	Krasnodarskaya		"KSY"	
	Without salt	With salt	Without salt	With salt
2	42	49	44	46
4	56	62	54	61
6	69	71	67	71
8	59	62	55	59
10	44	47	43	47
12	50	54	49	52
14	47	59	50	56
24	50	60	55	57
Average	52	58	52	56

Table 4. Loss in Weight During Fermentation by Flour-Leavened Brew (in g)

Duration of fermentation (in hr)	Strain			
	Krasnodarskaya		"KSY"	
	Without salt	With salt	Without salt	With salt
6	0.61	0.55	0.64	0.57
6	1.78	1.55	1.89	1.81
Average	1.20	1.05	1.26	1.19
Difference	0.15		0.07	



Yeast colonies. 1) "Krasnodarskaya" strain on unsalted medium; 2) "Krasnodarskaya" strain on a salty medium; 3) "KSY" strain on an unsalted medium; 4) "KSY" strain on a salty medium.

the resultant mixture a 20% solution of salt was added in such amounts as to produce a salt concentration of 0.8%; to the other retorts equivalent amounts of water were added. The retorts were kept in a thermostat at 30°. The loss in weight after 6 hr of fermentation is shown in Table 4.

The results in Table 4 show that the fermenting activity of liquid yeasts of the "KSY" strain when prepared with and without salt was greater than in yeasts of the "Krasnodarskaya" strain. Salt had an inhibiting effect on the fermenting activity of both yeast strains but its effect on "KSY" yeasts was slighter.

Thus our previous findings concerning the inhibiting action of salt on the activity of yeast cells and the greater fermenting activity of "KSY" yeasts have been corroborated.

In contrast to the assertions of Donchenko and Kuz'menko (1958) regarding the positive effect of salt on the growth and activity of yeasts of the "KSY" strain, it has been established that they, too, are inhibited by salt but to a lesser degree than yeasts not adapted to salt. Thus, the positive results obtained in the production of bread when salty liquid "KSY" yeasts were used cannot be explained by the favorable effect of salt on yeast. As these results were obtained in spite of the inhibiting action of salt on yeast cells they must, therefore, depend on other factors.

It has already been demonstrated (Roiter et al., 1959a) that the addition of salt to liquid yeasts has a negative effect on the fermenting activity of yeast cells and reduces their proteolytic activity as a result of which the hydrolytic effect of liquid yeasts on the dough

Table 5. Content of Water-Soluble Substances in the Medium as a Result of the Solution of Gluten by Yeast Cells (%)

Krasnodarskaya strain		KSY strain	
Without salt	With salt	Without salt	With salt
1.93	1.90	2.34	1.87

Table 6. Specific Volume and Porosity of the Bread

Qualitative indices of the bread	Krasnodarskaya strain		KSY strain	
	Without salt	With salt	Without salt	With salt
Moisture of the leaven 46%; specific volume in ml/g	2.72	2.61	2.65	2.82
porosity in %	73.7	72.9	72.9	75.0
Moisture of the leaven 70%; specific volume in ml/g	2.72	2.76	2.69	2.84
porosity in %	74.8	75.0	73.3	75.3

albumin decreases and its gas-retaining properties increase. Depending on the relation of the negative effect of salt on the activity of yeasts and its positive effect on the physical properties of dough, the quality of the baked bread either worsens or improves. That is why we verified the ability of yeasts of both strains to liquefy the gluten in the nutrient medium. Pure yeast cultures after 5 passages on a 12% malt wort (with 1% and without salt) were centrifuged in sterile conditions. The separated and thoroughly washed yeast mass was then diluted with water to 1/4 of its original volume. Three ml of a yeast suspension and 0.9 g of dry gluten was then introduced into a tube with a ground glass stopper. After a 24-hr incubation at 30° the mass in these tubes was centrifuged and in the centrifuge the content of water soluble substances was determined by a refractometer. A control test was arranged at the same time in which, instead of a yeast suspension, sterile distilled water was used. The number of water-soluble substances which had accumulated in the medium as a result of the action of the proteolytic ferments of yeast cells was determined by the difference in the refractive indices. From the results (Table 5) it will be seen that the "KSY" yeast cells cultivated on salt-free media had the greatest proteolytic activity.

In the investigation, laboratory test-bakings of bread were also made. The dough was prepared by the sponge method from type 1 flour, using liquid yeast in amounts of 25% of the weight of flour in the dough. The salt content of the dough was 1.3% of the total weight of the flour (or 0.8% of the weight of the dough), taking into account the salt in the liquid yeast. Sponges containing 46% moisture were prepared from 50% of the total flour, and 70% moisture from 30% of the total flour. They fermented for four hours. Dough containing 44% moisture was mixed. The duration of its fermentation was 1.5 hours. Small loaves of bread weighing 500 g, some of which were shaped by hand and others molded in bread pans, were prepared from the dough. Full rising time was allowed. The bread was baked for 25 minutes at a temperature of 230 deg.

According to the organoleptic indices and acidity, loaves which had been baked with different yeasts showed no differences. The relation of height to the diameter was greater in oven-baked loaves with "KSY" yeasts which had been cultivated on a salty medium. The porosity and specific volume of the bread are given in Table 6.

When liquid yeasts were prepared without salt, bread baked with "KSY" yeast was of a smaller volume and porosity than when yeasts of the "Krasnodarskaya" strain were used. The fact that the energy of fermentation in "KSY" yeasts is greater than in the "Krasnodarskaya" strain shows that "KSY" yeasts when cultivated without salt, thin out the dough more actively, reducing its gas-retaining capacity, and give a denser bread.

When bread was baked with "KSY" yeasts cultivated with salt, despite the inhibiting action of salt on the yeast cells, volume and porosity of the baked bread were greater than when the same yeasts without salt or yeasts of the "Krasnodarskaya" strain prepared without salt were used. This was due to the greater energy of fermentation of yeasts of the "KSY" strain and to the inhibiting effect of salt on their proteolytic ferments.

The data in Table 6 shows also that when bread is baked on yeasts of the "Krasnodarskaya" strain, the addition of salt has a negative effect on the quality of the bread only when dense leaven is used, but it does not worsen the quality when dough is prepared with liquid leaven. We also observed this in the course of an investigation of the conditions of production of dough on thick and liquid leaven. Thus, when liquid leaven was used the quality of the bread did not deteriorate when salt was added to liquid yeasts of the "Krasnodarskaya" strain and, on adding salt to the "KSY" yeasts, the quality improved.

SUMMARY

1. The addition of 0.8% salt during the cultivation of liquid yeasts inhibits the activity of yeast cells both of the "Krasnodarskaya" and "KSY" strains, but the latter are inhibited to a lesser extent than the former.

2. "KSY" yeasts exhibit certain peculiarities when compared with yeasts of the "Krasnodarskaya" strain. Among the by-products of fermentation they release more acids and have a higher energy of sugar fermentation and proteolytic activity.

3. Yeasts of strain "KSY" in comparison with yeasts of the "Krasnodarskaya" strain, when used for the preparation of liquid yeasts with 0.8% salt, yield bread of greater volume and of greater porosity, whereas in the absence of salt in the yeast the resultant bread is of inferior quality.

4. The addition of salt has an adverse effect on yeasts when dense leaven is used but a favorable effect on bread when liquid leaven is used.

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ANTIBACTERIAL PROPERTIES OF IODINE-POLYVINYL ALCOHOL

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At present, in medicine, high polymers are used mainly as blood substitutes and as plastic materials for surgical purposes. The possibility of the widespread use of certain currently produced high polymers as antiseptics has so far not been investigated. In the literature, there are only isolated, brief reports on the antibacterial properties of certain iodine-producing high polymers. The only one to have been adequately investigated is poly(vinylpyrrolidinone)-iodine (Shelanski and Shelanski, 1956; Siggia, 1957; Runti, 1957). According to these authors, poly(vinylpyrrolidinone)-iodine is a stable complex with pronounced antibacterial properties and slight toxicity. The preparation was successfully used in the treatment of bacterial and fungoid diseases of the skin and mucous membranes.

Mokhnach (1959), on the basis of the results of his investigations of the antibacterial, fungicidal, and curative properties of iodine-starch and its components, began a similar investigation of iodine-polyvinyl alcohol, which was produced as far back as 1926, by Frey and Stark (Staudinger, 1926), but little studied. It was assumed that this high polymer, like the other iodine high polymers, had antibacterial properties and that it was nontoxic for higher animals and man. It was also thought that it had an important advantage in that it could be administered parenterally, and, more specifically, intravenously and by endolumbar puncture. These assumptions have all been confirmed by experiments.

The present paper presents the results of an investigation of the antibacterial properties of iodine-polyvinyl alcohol.

METHOD OF INVESTIGATION

For the synthesis of iodine-polyvinyl alcohol, we used the polyvinyl alcohol preparation "NIIPP-32" (of the Leningrad Scientific-Research Institute of Polymerized Plastic Substances). It is a slightly yellowish powder completely soluble in water to a concentration of 9% inclusive, acid number 3.5 mg, content of volatile compounds 3.8%, acetate groups 0.75%, combined sulfur 0.15%, viscosity of the 8% solution 52.34 cp.

By the action of the calculated amount of molecular iodine in the potassium iodide solution (potassium polyiodide) on 1-2% water solutions of polyvinyl alcohol, iodine-polyvinyl alcohol solutions which contained the requisite amount of active iodine were obtained. These solutions were completely transparent and had the same deep blue color as the iodine-amylose solutions; they were also very stable even when kept for long periods.

The antibacterial activity of iodine-polyvinyl alcohol was determined in the following way. From 1-2% solutions, a number of dilutions were prepared with saline. To each dilution a bacterial suspension of 5 million cells prepared from a 24-hr agar culture of the experimental strain was added. After a 15-min exposure, from each dilution of the preparation, sowings were made on meat-peptone agar in Petri dishes. The results were recorded after an interval of 24 hr. Absence of growth indicated the complete bactericidal activity of the preparation; presence of individual colonies in the sowings at the time of continuous growth in the control indicated partial antibacterial action.

The study of the iodine-polyvinyl alcohol showed that in many respects, in its chemical, physical, and biological properties, this synthetic high polymer resembles the iodine complexes of natural high polymers, i.e., starch and amylose. The blue iodine reaction, the similarity of the absorption spectra, and the antibacterial activity lead to the assumption that the chemical structures of these complexes have certain common features. Judging by the literature data (Kramer, 1951; Lautsch et al., 1958), polyvinyl alcohol is classified with those high polymers which readily form inclusion compounds. On this assumption, the same structure could be ascribed to polyvinyl alcohol as has been accepted on the basis of Freudenberg's work in 1939 for iodine-amylose. Iodine molecules included in the polyvinyl alcohol chain form a single group with shared electrons drawn to the polymer molecule. Because of the fluctuation in the electron density, the electronic gas becomes a supply source of iodine ions which have strong oxidizing properties.

The pronounced oxidizing properties of the iodine ions explain the antibacterial action and the wide antibacterial spectrum of iodine-polyvinyl alcohol.

EXPERIMENTAL RESULTS

As the results presented in Table 1 show, iodine-polyvinyl alcohol has practically the same strong antibacterial action both on Gram positive and on Gram negative microflora. The antibacterial effect becomes

Table 1. Antibacterial Spectrum of the Action of Polyvinyl Alcohol Iodine

Test bacteria	Concentration of the preparation in γ/ml at which is observed:	
	Complete absence of growth	Partial inhibition of growth
Staph. aureus	12.5–25	–
Staph. albus	12.5	–
Strept. haemolyticus	12.5	–
Sarcina lutea	25	–
Escherichia coli commune	12.5–16	–
Escherichia coli (entero-pathogenic strains 0–26 and 0–111)	12.5	–
Salm. thyphi abdominalis	12.5	–
Sch. dysenteriae Sonne	25	12.5
Sch. dysenteriae Flexneri	25	–
Pseud. pyocyanea	25	12.5
Proteus vulgaris	12.5	–
Bact. prodigiosum	12.5	–
Bac. mesentericus	12.5	6
Bac. subtilis	12.5	–
Bac. mycoides	25	–
Bac. megaterium	12.5	–
Bac. cereus	12.5	6

Table 2. Antibacterial Activity of Polyvinyl Alcohol Iodine as Compared with Potassium Iodide

Test bacteria	Bactericidal concentration of iodine in solution in γ/ml	
	Polyvinyl alcohol iodine	Potassium iodide
Staph. aureus	16–32	64
Escherichia coli	16	16–64

manifest at concentrations of the preparation of 12.5–25 γ/ml following a 15-min exposure. In certain cases partial inhibition of growth was recorded at concentrations of 6 γ/ml. At no time did we record a decrease in the antibacterial effect of active iodine when it was included in the polyvinyl alcohol molecule compared with the action of potassium iodide with the same concentration of active iodine in the solution. In isolated cases a slight increase in the antibacterial activity of iodine was observed even when it was included in the polyvinyl alcohol molecule (Table 2).

On decolorizing the iodine-polyvinyl alcohol solution by the action of alkali, the antibacterial properties disappeared altogether and were not subsequently restored. In this respect, it is very similar to iodine-amylose and iodine-starch. In both cases the decolorization of the complex with simultaneous loss of antibacterial properties is explained by the action of the alkali, which causes the positively charged ion I^+ to change into the anion I^-. Moreover, the coloration center formed by the positively charged ion or polarized iodine molecule is destroyed, and the antibacterial properties disappear.

SUMMARY

1. Iodine-polyvinyl alcohol has pronounced antibacterial properties and a wide spectrum. It has practically the same strong effect on Gram-positive as on Gram-negative organisms.

2. Iodine shows no reduced antibacterial action when incorporated into the iodine-polyvinyl alcohol molecule.

3. When iodine at certain concentrations is incorporated into the molecule of polyvinyl alcohol, it exhibits a certain increase in antibacterial activity as compared with the control solution of potassium iodide.

4. Iodine-polyvinyl alcohol solutions lose all their antibacterial properties when subjected to decolorization.

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A SIMPLE FILTRATION APPARATUS FOR DIRECT COUNT OF BACTERIA ON MEMBRANE FILTERS

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Counting of bacteria by the direct count method is finding more and more application in various microbiological investigations, both in those conducted for purposes of scientific study and in those aimed at solving purely practical problems (Razumov, 1932, 1947, 1955; Yakovenko, 1950; Alfimov, 1954; Meshkov, 1958).

Kopp (1941), in his description of ten different procedures for the quantitive determination of bacteria in water by the direct count method, notes that they are all modifications of the following two steps: 1) concentration of the microbes from various volumes of water and preparation of the materials, and 2) staining of the preparations and computation of the bacterial cells.

The most convenient method for concentrating microbial cells appears to be filtration of specified volumes of water through membrane filters. Included as a part of this concentrating procedure is Razumov's method for the direct count of bacteria (1932, 1947), which has now been more fully and systematically worked out.

For filtration of water through membrane filters, a series of apparatuses has been proposed which Razumov describes in detail in his survey (1955). All these apparatuses, including even the simplest of them, Pel'sh's clamp No. 1 (1935), can be constructed either in the industrial laboratory or in the experimental workshop. However, special receptors for the filtrates and various pumps (Kamovskii's waterjet pump) are indispensable for work with these apparatuses.

We have undertaken to design a simple apparatus for water filtration which can be used for the direct count of bacteria. A description of this apparatus is here presented. *

The structure of the proposed apparatus is shown in Figs. 1 and 2. Figure 1 shows the apparatus in its assembled form.

The apparatus consists of a "Rekord" syringe with needle, a segment of a glass test-tube, a rubber stopper, and a hollow glass stopper which serves as the stand.

The apparatus is constructed in the following way: in the rubber stopper (Fig. 2, 1) cylinder 3, 10 mm in diameter, is cut out by means of a cork borer. With the removal of cylinder 3, canal 2 is formed in rubber stopper 1. Into cylinder 3, which has now been removed from the stopper, the transverse canal 4 is drilled perpendicular to the long axis. For this, a drill must be chosen which is of the right size to give canal 4 a diameter of approximately 5 mm. Canal 4 should be located 3–4 mm from base 5 of cylinder 3. With a

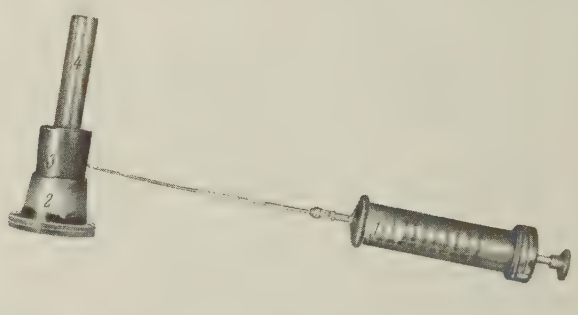

Fig. 1. The structure of the filter apparatus in its assembled form. 1) Syringe with needle; 2) stand; 3) rubber stopper; 4) glass for water (segment of test-tube).

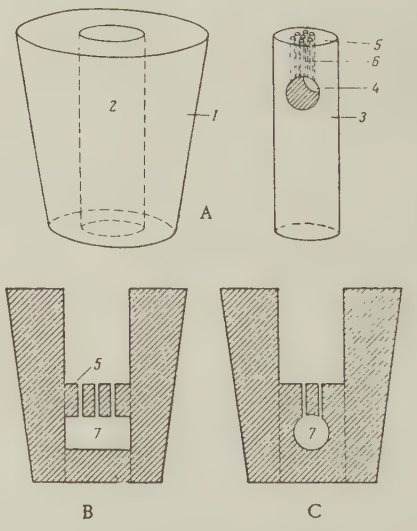

Fig. 2. The structure of the filter apparatus when dismantled. A) External view; B) longitudinal section of chamber 7; C) cross-section of chamber 7; 1) Rubber stopper; 2) canal formed by removal of cylinder; 3) cylinder; 4) transverse canal in cylinder; 5) upper base of cylinder; 6) passages connecting base of cylinder to transverse canal; 7) chamber to be pierced by needle of syringe.

*We wish to express our gratitude to Prof. V. A. Yakovenko for his many useful suggestions during the construction of this apparatus.

heated needle or a sharpened wire several (4–5) short passages are cut through at base 5 to connect base 5 with canal 4. Then cylinder 3 is reinserted into canal 2 of stopper 1, but, instead of being replaced in its original position, this time base 5 should face in the direction of the top end of stopper 1. Cylinder 3 should be inserted into canal 2 to approximately half the height of stopper 1. Owing to the transversal canal 4, chamber 7 (Fig. 2, longitudinal section B) is thus formed within the thickness of stopper 1, and this chamber is then pierced by the needle of the syringe. Stopper 1 is now fixed onto the stand (Fig. 1). The hollow glass stopper which is to serve as the stand should be one into which rubber stopper 1 fits only with some effort. Rubber stopper 1 should be inserted into the stand as far as it will go, so as to assure that the part of cylinder 3 which does not enter canal 2 rests on the bottom of the hollow glass stopper. After stopper 1 is secured into the stand the syringe is then attached to the needle.

The glass container (Fig. 1) into which the water for filtration is poured is prepared in the following manner: an ordinary chemical test-tube is cut transversely in the same way as in cutting glass pipes (Voskresenskii, 1956). The part of the test-tube which forms a cylinder is used as the water container. The test-tube used for this should be one which can be inserted into canal 2 only with some effort.

Filtration of water with this apparatus takes place in the following manner: membrane filters are cut with a diameter corresponding to that of the canal at base 5 (Fig. 2), and with the aid of tweezers, they are laid over the surface of base 5. The glass cylinder then is inserted into canal 2 as far as it will go (Fig. 1), so that its edges press the membrane filter flush against the surface of base 5. Owing to the elasticity of the rubber stopper, the walls of canal 2 hug both the inserted glass cylinder and cylinder 3 with sufficient tightness to guarantee the hermetic sealing of the system. The water to be studied is then poured into the glass cylinder. Filtration of the water through the membrane filter takes place owing to the vacuum created by the syringe. The syringe serves two purposes at once—it creates a vacuum in the system and acts as receptor for the filtrate.

After filtration of the specified volume of water, the glass cylinder is withdrawn from canal 2, and the membrane filter is removed with tweezers from the surface of base 5, to be processed later on according to Razumov's method (1932, 1947).

No more than 20–30 minutes is consumed in assembling this simple filter apparatus. When dismantled it can be packed into the small sterilizer made especially for the "Rekord" syringe.

This filter apparatus makes it possible for the concentrating of bacteria on membrane filters to be done immediately at the cistern or reservoir where the water sample is taken.

SUMMARY

A description is presented of a simple filter apparatus for direct count of bacteria on membrane filters.

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PRESERVATION OF MOLDS

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Conidia (asexually produced spores) are of primary importance in multiplication of molds used in manufacture of citric acid, fermented substances, antibiotics, and so forth.

Conidia of representative species of Aspergillus and Penicillium remain viable for long time intervals after dehydration; in moist environment they survive 95°–98°, and up to 130° (Panasenko, 1944) when treated with dry heat. Kursanov (1940) mentions that conidia of Penicillium glaucum remain viable for 2 years and those of Cladosporium herbarum, for up to 10 years. McCray was able to preserve spores of Aspergillus oryzae for 20 years (cited by Protod'yakonov and Palei, 1937).

We have data pertaining to preservation of mold cultures held without transfer for 5 years.

The mycological collection of the Ukrainian Scientific Research Institute of Food Technology was not transferred during World War II. The temperature in the unheated building reached −30° during the winter and rose up to +33° during the hot summer days. Only one third of the molds of the collection did not survive these unfavorable conditions. However, by no means did all molds develop following the initial transfer upon fresh nutrient media after a lapse of five years.

Many strains had to be "revived", according to Naumov's technique (1937).

Of primary importance among the surving molds were all the species of genus Aspergillus, of which only certain strains of A. sulphureus and A. conicus, as well as several strains of A. ochraceus, were lost. Generally speaking, species of the genus Penicillium, present in large numbers in the museum, also survived, but primarily at the expense of the more widely distributed strains. It has not been possible to "revive" the species rarely found in our environment, such as P. colitum, P. Ehrlichi, P. Thomi, P. canescens, P. spinulosum, P. psittacinum, P. melinnii.

Certain other Fungi Imperfecti also did not survive unfavorable conditions; lost were all strains of Monilia sitophila, Botrytis cinerea, Cladosporium herbarum. Certain mucoraceous molds were most seriously affected: Mucor racemosus, Rhizopus nigricans, Thamnidium elegans.

Certain strains of the surviving fungi degenerated. This degeneration was expressed in changes in morphological individuality of cultures, in changes of sizes and form of sporangiophores and conidiophores, in sharp reduction in the number of spores or complete loss of spore formation, degeneration of cellular mycelium evident by the appearance of large numbers of oily droplets, and development of slimy substrate mycelium.

Most strains preserved their cultural and morphological characteristics. Changes in certain strains developed at the expense of formation of hirsute forms; in others there were formed folded rhizomes.

Such fungi as P. luteum and P. spiculisporum lost their spore-bearing asci but retained the ability for conidial sporogenesis. A similar phenomenon is observed after prolonged cultivation of Aspergillus with an ascous stage. Spore-bearing asci disappear relatively rapidly, while the conidial sporebearing becomes more intense.

A yeast collection that had not been transferred for 5 years survived almost in its entirety.

These chance observations, although of considerable interest, cannot be utilized in practical preservation of industrial cultures.

For industrial operations it is important to preserve not only the viability of spores, but also the biochemical activity of strains.

Statements in the literature point out that frequent transfers of molds lower their biochemical activity (Protod'yakonov and Palei, 1937). According to our results, the depressed biochemical activities of cultures forming less active variants is equally possible from either frequent transfers or from intermittent transfers with prolonged intervals. These cases are influenced by the phase-of-growth curve used for routine spore transfer.

Thom (1939) reminds us of two strains of A. niger which were preserved in his laboratory for five years without morphological alteration.

Species of Aspergillus and Penicillium are ephemeral plants and, under favorable conditions, complete their growth cycle in 15 days with conidial sporogenesis and in 25 days with formation of the ascous stage. At this time, the spores are completely formed and, because of a strong outer membrane, are prepared to survive unfavorable conditions. In preservation of molds under laboratory conditions, without influence of strongly active factors, one seldom observes the appearance of new forms.

The problem of preservation of mold spores is considered adequately solved by application of lyophilization. Drying under vacuum without cooling als

showed adequate results. According to Novoselova (1955), ripe conidia of A. niger retain up to 70% of their viability for 1–2 years, and their ability to form acid is indistinguishable from that of the original culture.

It is necessary to stipulate that any procedure for dehydration of mold spores can be made effective, when one takes into consideration the known characteristics of cultures.

The duration of preservation of the inoculated material and of viability of spores of stock cultures are of considerable importance in manufacture of alcoholic products and of citric acid. As a result of extensive experience in these industries the period for preservation of stock cultures of A. niger and A. oryzae has been set at 3 to 4 months (Instructions, UNIIPP, 1956; Manufacture of Edible Acids, 1953). The incubation time for inoculum is determined by the technology of the respective industry.

In industrial production of citric acid, A. niger is grown for 5–6 days in special flasks on liquid nutrient medium and spores are removed from the gradually dried surface films by means of a suction pump. These dehydrated spores are preserved for up to two years (Manufacture of Edible Acids, 1953).

In preparation of the inoculum for fermenting reactors, A. oryzae is grown for 3–4 days in cuvettes on bran medium. Then the contents are dehydrated to 20–25% of the initial moisture and preserved for up to two weeks (Instructions, UNIIPP, 1956).

In the course of our studies of conditions for preservation of biochemical activity of molds used in the manufacture of fermented products and citric acid, we grew A. niger and A. oryzae on different nutrient media, and also preserved our experimental cultures dried in vacuum for different periods of time. The stock cultures were also checked. We grew the experimental cultures on grapewort agar, on Czapek's medium to which were added certain carbohydrates, as well as on grains of different cereals with added soil. After incubation, the cultures were kept at from 1° to 5°. Conidia, separated from mycelium, were kept either in sand or without sand.

As a result of our investigations we concluded that growing molds on certain substrates could not possibly improve or fix their biochemical properties. Changes in morphological, cultural, and biochemical properties of molds depend on conditions used in their cultivation. The variability is evident in the entire colony of the mold.

In formation of stable variants, only a certain portion of a colony, such as a sector, a ring, or center, may be subject to change. It is possible to preserve given properties of any culture when we know the nature of its hereditary variability. It is necessary to select conditions for preservation of each strain in keeping with the characteristics of its growth and variability. The viability of mold spores is of considerable importance for the stock and industrially used cultures.

In obtaining cultures from single spores by means of micromanipulator, we paid attention to the inadequate conidial development in certain strains. Ordinarily, we used 20–30-day-old cultures for isolation of single spore preparations. Frequently, the spore membrane would become invaginated as a result of contact of the dissecting needle with the spore in question, and the spore would assume the shape of a pressed-in rubber ball. It is simple to pick up such spores with a dissecting needle; however, they do not germinate in a moist chamber. It appears as though these spores are empty. A poor degree of spore germination is also observed in other molds. Kursanov (1940) indicates that field mushroom spores germinate much more easily when grown as populations than when grown singly. In this connection he suggests the hypothesis that bios is required for the germination of the spores of the field mushroom and that, most likely, a single spore has an inadequate amount of bios, but taken as a group, spores enrich the medium with this agent and make it suitable for germination of at least a few spores.

The poor rate of germination of young conidia in certain representative species of Aspergillus and Penicillium evidently depends upon other factors. It is possible that uneven distribution of cellular elements takes place in formation of spores (conidia), and that certain conidia turn out to be nonviable. We observed the viability of mold spores kept under laboratory conditions for one and a half years, by counting the germinating spores. The number of germinated and ungerminated conidia was determined by the following method: The conidia were immersed in sweet beer wort of 6° Balling, and then 1 ml aliquots of this suspension were placed into Petri dishes. Spore germination was carried out at 22–30°. For counting, open Petri dishes were placed on a microscope stage. The number of germinated and ungerminated spores were recorded for each field of vision. Spores were counted after 4, 6, 8, 10, 12, 14, 16, 20, and 22 hours. A. niger and A. oryzae germinated better at 30°; Penicillium, at 22°.

An attempt to enumerate the germinated spores in Goryainov's chamber was not successful, since the swollen and germinated spores, being quite large, clog the pipette and can not be observed in the camera.

Results different from those obtained by means of the surface method were observed in spores germinating submerged in sweet beer wort. A lower percentage of spores germinated in submerged state.

As can be seen from the table, various strains of the same species differ in viability.

Although the culture is stated in the table, it should be noted that there is a difference in the time required for formation of conidia in different strains. Thus, in P. roqueforti, P. chrysogenum, A. niger, and A. oryzae with smooth growth, spore formation takes place in 3–4 days after inoculation, but there is a considerable delay in formation in rough forms, and in a strain of A. oryzae with fluffy growth spore germination is initiated in 8–12 days.

It is of interest to note that in strains with low degree of initial spore germination, as in A. oryzae 81, A. niger 472 and 473, and P. roqueforti 335–366, the germination subsequent to extended preservation is

Preservation of Viability of Mold Spores Kept Under Laboratory Conditions

Molds	Strains	Germination of spores (%)			
		15 days	1 month	6 months	18 months
A. oryzae	81	84	80	55	20
	476	100	100	99	79
	378	100	100	70	43
A. niger	20	100	100	100	80
	72	100	100	70	68
	228	100	100	99	76
	472	98	92	66	53
	473	95	91	67	64
P. roqueforti	335	74	57	33	33
	366	71	46	38	30
P. chrysogenum	376	100	82	67	53
	377	92	81	63	51

lowered more rapidly than in cultures having high initial germination rates.

In addition to preservation of viability, the time required for spore germination after inoculation is of considerable industrial importance. Spores of young cultures germinate synchronously and rapidly in 4–6 hours. Spores of old cultures germinate considerably slower and develop at random, some in 8 hours, others in 14–22 hours.

Our strains with high saccharolytic activity are also noted for rapid mycelial formation. Thus, in 3 hours, mycelium of A. oryzae kept at 28° grows 52 μ, while in the same time mycelium of a strain of A. oryzae 81 only succeeds in growing 20 μ. Such properties of a mold may be of considerable industrial importance. In the case when there are two molds with similar biochemical properties, but differing in the rate of growth and spore germination, it is clear that it is more advantageous to use the strain with synchronous spore development and more rapid mycelial growth. It is simpler to use such a strain in the laboratory and in industry. Under industrial conditions this strain completes its growth cycle more rapidly and is less subject to contamination.

SUMMARY

1. The methods for storage of molds should be selected in accordance with the hereditary variability of the strains.

2. Counts of germinating spores should be made directly in Petri dishes.

3. Viability of mold spores, even when stored under identical conditions, varies in different strains of the same species.

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DISCUSSION

THE STABILITY OF PHYSIOLOGICAL CHARACTERISTICS AND THEIR SIGNIFICANCE IN CLASSIFICATION OF ACTINOMYCETES

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Standardization of procedures, as well as the establishment of most suitable characteristics and reactions, are of considerable significance in determination and description of actinomycetes. To this time, it is not clear which of the many qualitative characteristics of differing diagnostic significance introduced in descriptions of species are to be considered as primary and which are secondary, serving only for more detailed characterization of special strains.

One should consider the variability of an organism, its capacity to yield different variants. As is known, organisms have specific properties, and it is very seldom possible to isolate identical cultures. This variability is explainable by genetic differences in qualitative properties of different sections of the mycelium and spores.

Characteristics of the actinomycetes are usually grouped into 3 categories: morphologic (structure of the sporophores and sphores), cultural (color of aerial or substrate mycelium; presence of soluble pigments), and physiological or biochemical (fermentation of milk, gelatin, starch, nitrates, sucrose). The stability, as well as variability, of these characteristics differs and depends upon conditions used for preservation of cultures, as well as experimental conditions and properties of the organism. It is known that there are stable species with clearly expressed stable characteristics, as well as variable species. It is now established that morphological characteristics are subject to less variation and biochemical characteristics to most variation.

This study is devoted to elucidation of physiologic variability of actinomycetes as determined by the time of storage of stock cultures as well as to the evaluation of the diagnostic value of these characteristics.

Cultures of different species of actinomycetes have been a part of the collection of the Institute for the Investigation of New Antibiotics for many years. We studied the stability of characteristics in cultures stored on agar slants. Twice a year the strains were transferred on Gauze's mineral medium 1 and organic medium 2, allowed to develop for 10–15 days at 28°, and then stored at room temperature. The duration of storage varied from 4 to 7 years. In 1954–55 a most complete investigation was made of cultural and physiologic characteristics. In 1959 the complete listing of properties was carried out for the second time.

In the present communication we discuss only the cultural and physiological properties of cultures as they develop in milk, gelatin, starch, nitrates, sucrose, and cellulose. Investigation of these characteristics was carried out by routine methods.

Eighty-nine species of actinomycetes represented by 371 cultures were studied. Strains with pale yellow, white, rose, gray, and blue aerial mycelium were among the species in question.

There was no significant change, after storage, in cultural characteristics of actinomycetes on media used for determination of biochemical properties; certain changes were observed only in the intensity of pigmentation of colored forms. A different picture was observed in physiological properties: Not a single reaction remained unchanged.

As can be seen from Table 1, half of the cultures changed their reaction in milk; in this all groups of actinomycetes changed in somewhat the same manner. As for series of cultures (Table 2), the cultures of some series changed more, of other series less, but this difference in most cases is not too great.

The least change on milk was shown by series Fradiae and Chromogenes (16 and 23%), the greatest change by series Albosporus and Roseoviolaceus (80%). Percent of changed strains of other series is somewhat the same.

The behavior of strains within the species is most diversified in relation to their activity in milk: Twenty species did not change in this property; however, all strains of 12 species changed, and of the remaining 57 species some changed and others did not.

As can be seen from our data, this is a relatively constant characteristic in many species of different series. Species without a single strain retaining its original characteristic varied in a different manner: Strains of Actinomyces albogrisedus (series Griseus), as well as strains of Actinomyces coeruleofuscus (series Coerulescens), initially differing in activity, became identical; strains of Actinomyces glaucescens (series Coerulescens) retained their individuality; three identical strains of Actinomyces venezuelae var. spiralis (series Lavendulae–roseus) varied in a different manner; strains initially of common origin and

Table 1. Variability of Physiological Properties of Actinomycetes on Different Media (% of total number of strains of the group)

Group of actinomycetes	Total no. of strains	Strains not changed %	Strains showing variation						
			Total %	In milk	In gelatin	In starch	In cellulose	In nitrates	In sucrose
White and pale-yellow	81	31	69	40.7	7.4	6.6	19.7	9.8	14.8
Rose	104	27	73	54.8	4.8	20.2	17.3	6.7	13.4
Gray	163	18	82	46.6	12.3	19.0	20.1	20.6	23.0
Blue	33	3	97	57.6	9.6	24.2	57.6	15.1	39.3

Table 2. Variations in Physiological Properties by Actinomycetes of Different Series

Group	Series	Number of strains	% strains showing variations on					
			Milk	Gelatin	Starch	Cellulose	Nitrates	Sucrose
White and pale yellow	Helvolus	51	13.3	0	4.0	25.5	15.7	13.7
	Albus	19	40.1	0	0	10.5	0	26.3
	Albosporeus	11	81.8	9.0	9.0	9.0	0	0
	Lavendulae roseus	42	52.0	9.5	16.6	16.6	2.4	4.7
	Fradiae	12	16.6	0	25.0	41.6	0	75.0
Rose	Fuscus	27	59.0	0	3.7	7.4	0	0
	Roseoviolaceus	15	80.0	0	60.0	20.0	27.0	7.0
	Ruber	8	62.0	12.5	12.5	12.5	25.0	25.0
	Aureus	27	48.1	7.4	3.7	11.1	33.3	22.2
	Violaceus	35	54.3	17.1	14.3	11.4	23.0	20.0
Gray	Chromogenes	17	23.5	6.0	35.3	41.2	6.0	35.3
	Chrysomallus	23	47.8	8.7	26.0	41.7	39.1	26.0
	Griseus	61	47.5	14.7	21.3	16.4	26.2	19.7
Blue	Coerulescens	33	57 6	9.9	24.2	57.6	15.1	39.3

Table 3. Variations in Physiological Properties of Different Species of Actinomycetes (Expressed as % of Total Number of Species of a Group)

Groups	Total number of species	Strains not changed, %	Variations in growth on					
			Milk	Gelatin	Starch	Cellulose	Nitrates	Sucrose
White and pale yellow	20	0	66	15	10	50	25	25
Rose	19	10	98	15	50	50	25	66
Gray	42	7	80	35	50	40	56	58
Blue	8	0	75	25	50	87	25	75

initially similar members of seven species of different series varied, but after variation, they remained similar.

We were unable to establish any connection between the activity in milk and membership of the species in one or the other series. In most cases, there is no relationship of this nature between strains of one species. Extensive variation in activity in milk is inherent in most of the actinomycetes, and therefore we believe that one should not ascribe any diagnostic significance to coagulation or peptonization of milk. Most likely the ability to change milk one way or another is a property of a strain, and not of a species.

Properties such as hydrolysis of starch, growth on cellulose, reduction of nitrates, inversion of sucrose and liquefaction of gelatin did not vary as intensely as activity on milk (Table 1). The properties of pale yellow, white, and rose actinomycetes turned out to be quite stable, while gray and especially blue were subject to extensive change. If we should consider less inclusive groups (series), then we can note that among the pale yellow and white actinomycetes members of series Helvolus were most variable; among the rose, series Roseoviolaceus and Fradiae; among the gray, Chrysomallus (Table 2).

The series Chromogenes changed essentially on account of the species Actinomyces variabilis var. roseolus; two species did not change at all. Only 5 of the 89 species retained all characteristics without changes: Two species from the rose group (Actinomyces umbrinus and Actinomyces cinnamonensis var. proteolyticus) and three species from the gray groups (Actinomyces griseoincarnatus, Actinomyces fumosus, and Actinomyces flavochromogenes).

All the other species varied to a greater or lesser degree and in different ways (Table 3).

Thus, 10% of the white and pale yellow species and 50% of the species of the other groups changed their activity in starch; most variability in nitrates was due to the gray species (about 56% of all species) and when tested on cellulose and sucrose the blue species were involved (87% and 75% respectively).

In this manner, one and the same characteristic varies in different ways in different groups.

As for strains within one species, their qualities change independently of each other and independently of the position of the species. Only 3 entire species changed in behavior on starch, 2 species on sucrose, 4 on cellulose, and 1 on nitrates. As a rule, characteristics of individual strains of a species vary independently without any connection with other properties. Most often, variations take place on one or two media, less on three, and very seldom on four or five media, primarily in gray and blue actinomycetes which are more subject to variation.

Proteolytic enzymes are widely distributed among the actinomycetes and almost all liquefy gelatin. The relationship of actinomycetes to gelatin is a relatively stable characteristic, and according to our data, only 8% of all strains varied in this property. Four strains of Actinomyces griseoruber began to show good liquefaction, and four strains of Actinomyces gobitricini lost this property. Other cultures with altered activity were distributed more or less equally between species of various series.

Also unchanged were the growth characteristics on gelatin; specifically, the color of substrate mycelium and the presence of pigments. Although the property of gelatin liquefaction is a stable characteristic, such widely distributed property can hardly serve as a good diagnostic characteristic.

In this manner, almost all physiological properties of actinomycetes change during storage of cultures. The most frequent change pertains to the activity on milk; the least frequent deals with the activity on gelatin. We were unable to establish any natural regularity in the character of these variations. As a rule, the strains vary independently of their taxonomic position. One did not see identical variability in all strains of a species on all media. Identical variability of the entire species on any one medium constitutes a rare event. As a rule, the characteristics vary independently.

Variability in characteristics pertaining to gross morphology of a culture was quite insignificant, being more qualitative than quantitative in nature. This allows one to speak of greater stability of morphologic characteristics in comparison with the physiological.

In connection with the material presented above, one questions whether it is warranted to trust, and if so, to what extent, the physiological properties of actinomycetes not only in description of species, but also in classification of a culture.

This point is of interest to many investigators studying taxonomy of actinomycetes since all of them encountered instability of these reactions. Krasil'nikov (1949) wrote that one must keep in mind the variability of physiological characteristics in general and the extensive variability of actinomycetes in particular in attempting to use them in taxonomic studies.

Waksman (1950) stated that the ability to utilize sucrose is indigenous to most actinomycetes, but that it is not always possible to prove the formation of invertase by these cultures, which fact lowers the value of this characteristic.

Waksman (1950) and Ocami (1952) consider that it is not possible to utilize liquefaction of gelatin as a characteristic for classification of actinomycetes since, with but a few exceptions, they are capable of this liquefaction.

Shinobu (1958), having studied extensively the utilization of different characteristics in taxonomy, concluded that action in milk, gelatin, sucrose, and development on cellulose can hardly be considered as useful characteristics. They are not constant, and experimental results are frequently contradictory. The author points out the stability of ability of actinomycetes to reduce nitrate. According to our data, this characteristic may also vary, and it may not be any better than reaction on sucrose or on starch.

Ettlinger, Hutter, Corbaz, et al. (1958) concluded that reaction in milk, liquefaction of gelatin, and hydrolysis of starch are all properties suitable only for characterization of individual strains. Variability in behavior in milk may differ so much in one and the same culture that they offer to completely discard this characteristic.

Baldacci said, during the Stockholm Conference (1958), that it is not known how many of the so-called biochemical characteristics utilized in classification of actinomycetes were introduced in classification in accordance with its needs and requirements. More likely, they were introduced by workers from other areas of science.

We agree with the opinions expressed by these investigators. It is always necessary to consider the variability of these characteristics, and it is impossible to single out a new species differing only in its fermentation activity from the one described before.

As for the phenomenon of variability of physiological reactions, it is entirely explainable and is not accidental in nature. The conventional methods for determination of activity of actinomycetes only consider the presence or absence of corresponding enzymes in the surrounding medium, and then only in products of dissociation, but do not determine in any manner the general presence or absence of the enzyme in the microorganism.

It is known that all the considered enzymes are adaptive in nature, and their formation depends on the composition of the medium and conditions of cultivation.

Thus, there is hardly a strain inactive on starch. Actinomycetes grow very well on starch medium; consequently, they utilize it, but not all secrete the amount of amylase in the surrounding medium adequate for the available rough methods of its determination. There apparently exists a well-known contradiction: A strain grows very well on a medium with starch as the only source of carbon, but no hydrolysis of starch occurs. This also takes place with other biochemical reactions. Actinomycetes grow quite well on solid as well as on liquid media with sucrose, but not all invert it. In reality, we determine not the presence of an enzyme, but the presence in the medium of products of sucrose hydrolysis which are utilized at different

rates. The amount of enzyme secreted in the surrounding medium apparently depends upon different conditions which are difficult to take into account.

From our point of view, cultural characteristics on certain media used for determination of biochemical reactions are more valuable for diagnostic purposes because of their greater stability. Cultural characteristics express an entire series of reactions, apparently of a more general nature than secretion into the surrounding medium of one or two enzymes.

SUMMARY

1. A study was carried out of the stability of physiological (biochemical) properties of 89 species of actinomycetes, represented by 371 cultures.

2. Physiological characteristics vary in time independently of each other and of the taxonomic position of the culture.

3. Most variable is the ability to react with milk; least variable is liquefaction of gelatin.

4. In contradistinction to the physiological properties, cultural characteristics of the species under investigation showed hardly any change on these media.

5. Proteolytic activity on milk and gelatin has no practical significance for classification of actinomycetes. Other reactions (hydrolysis of starch, growth on cellulose, inversion of sucrose, reduction of nitrates) are of doubtful significance in this connection. In a relatively small percent of strains that varied, only 5 of the 89 species retained all physiological characteristics unchanged. Any combinations of altered and not altered characteristics are possible within a single species.

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A CONTRIBUTION TO THE RATIONAL CLASSIFICATION OF ACTINOMYCETES

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More than eight decades have gone by since actinomycetes began to be studied. During this period of time, a tremendous amount of data has been obtained from this field of microbiology; in the first half of this period, the data have dealt chiefly with parasitic actinomycetes, while in the second half, with the saprophytic ones. At the end of the fourth decade, the fact that saprophytic actinomycetes produce antibiotics was definitely established (Krasil'nikov, Waksman, et al., 1938), as the result of which a whole series of "new species" of these microorganisms was described. Existing classification schemes proved to be inadequate for the systematization of such a large number of isolated strains, and the necessity arose for the creation of new classification systems for this group of microorganisms. In the last edition of Bergey's Manual (1957), about 150 species of actinomycetes of the genus Streptomyces alone are described, while for the genus Nocardia Trevisan—about 50 species. Meanwhile, in Krasil'nikov's key (1949), about 50 species of the genus Actinomyces (Streptomyces) are described, but then, about 150 varieties and strains are mentioned separately.

In view of such a situation, an urgent need has arisen to revise the principles of classification of actinomycetes and to create a rational system of classification of these microorganisms. Conferences in various countries (in Italy, USA, 1958) and international conferences (symposium in Rome, 1953; conference in Stockholm, 1958) have been devoted to this question. After the Stockholm conference, studies were conducted in a number of countries on certain properties of actinomycetes, using the same methodology and the same strains for the purpose of approving a single method on which their classification could be based. Since a discussion has been raised on the pages of the journal "Mikrobiologiya" concerning the problem of classification, we should like to express our opinion on the creation of a rational system of classification of actinomycetes.

It seems to us that it is necessary to dismiss the idea of species in microorganisms as being only a taxonomic concept (as is regrettably thought by the majority of microbiologists) and to adapt a new idea, shared by the minority of microbiologists (Krasil'nikov, 1945; Teshich, 1957; Baldacci, 1959, and others), that biological species are a reality with their numerous varieties and biological types as forms of existence, and that these species differ qualitatively among themselves in a whole series of characters. In addition to this, it is necessary to establish a broad system of classification of super- and subspecies of actinomycetes with a more precise determination of the hierarchical order of their characters. Morphological characters, which are the more stable, should be used as the basis for determining the higher systematic categories; physiological characters, which are less stable, should be used for the separation of the lower categories, while biochemical characters, which are the most variable, should be the basis for the classification of biotypes and strains of individual species. A large proportion of microbiologists now agree with these principles of classification, but frequently deviate from them when classifying actinomycetes. In no case can certain ecological characters (for example, relation to temperature, oxygen, or host) be regarded as a criterion for creating genera as is done by Waksman (1959), Henssen (1957), and others, since they are reflected in other characters of microorganisms which are growing under particular ecological conditions in nature.

In compiling our system of rational classification of actinomycetes, we utilized earlier existing systems (Krasil'nikov, 1949; Negroni, 1953; Baldacci, 1959), striving, on the one hand, to base the higher systematic units on the more constant morphological characters, while on the other hand, to avoid naming genera for individual scientists, which leads to etymological nonsense. In establishing endings for the different systematic categories, we also had the international codes of botanical and microbiological nomenclature in mind, and did not introduce a single new genus name for actinomycetes. Many old names were given a different significance in our classification system, but in this way, it was possible to avoid the use of synonyms. The new classification system, somewhat augmented in comparison with that proposed earlier (Teshich, 1959), is given below.

All actinomycetes belong to the class Actinomycetalia (Teshich, 1959), including those microorganisms which do or do not form mycelium in young cultures, which concurs with Krasil'nikov's definition (1949), although it seems to us that his name for the class (Actinomycetes) is not entirely equivalent, since Lachner-Sandoval (1898) used it as a common name for these microorganisms, and it designates the plural

of Actinomyces. This class is divided into two subclasses: 1) Mycobacteriineae (Teshich, 1959) to which microorganisms which do not form mycelium in young cultures belong, and 2) Actinomycetineae (Teshich, 1959)—actinomycetes which form mycelium in young cultures. We divided the second subclass into two orders according to the stability of the young mycelium. The order Proactinomycetales (Teshich, 1959) includes primitive actinomycetes with septate mycelium which breaks up into separate segments. To it belongs the family Proactinomycetaceae (Lehmann et Neumann, 1927, emend. Teshich, 1959), since it formerly included the group of microorganisms which we set apart in a separate subclass as a transitional group between true bacteria and actinomycetes. This family had not been divided into genera, although attempts in this direction existed (Jensen, 1953), and we made this division. Krasil'nikov (1938), Umbreit (1939), and others distinguished two natural groups of primitive actinomycetes which differed in their ability to form aerial mycelium ("Sporophores"). We divided these into two separate genera in order to avoid synonyms relating to the same microorganisms.

The genus Proactinomyces (Jensen, 1931; emend. Teshich, 1959) includes primitive actinomycetes which do not form aerial mycelium, or "sporophores" (Jensen, 1953), and includes Krasil'nikov's (1938) and Umbreit's (1939) first group. Bergey described 24 such species, and Krasil'nikov (1949)—about 14 species. The species P. farcinicus (Nocard, 1888) was the earliest to be defined.

The genus Euactinomyces (Langeron, 1923; emend. Teshich, 1959) includes primitive actinomycetes which form aerial mycelium ("sporophores") and forms which approach the true actinomycetes. According to Jensen's classification (1953), representatives of Krasil'nikov's (1938) and Umbreit's (1939) second group are included here, although the Danish microbiologist suggested the old genus name—Nocardia (Trevisan, 1888)—for them. There are many reasons for leaving the first name of the genus according to the proposal of the French microbiologist to replace the old name of the genus Actinomyces (Harz, 1877); however, in this case, the principle of priority would not be taken into consideration, since a culture of these microorganisms was first obtained by Israel (1884), and only later by Nocard (1888). The difficulties of using various synonyms would disappear with the acceptance of the proposed name. Bergey (1957) describes 21 species of this type, while Krasil'nikov (1949) has 19 species, of which E. caprae (Silberschmidt, 1889) is the oldest of all.

The order Actinomycetales (Buchanan, 1917; emend. Teshich, 1959) includes true actinomycetes with nonseptate stable mycelium, which form secondary aerial mycelium in the majority of cases. The order is divided into three families according to the arrangement of spores.

The family Micromonosporaceae (Krasil'nikov, 1941) includes true actinomycetes in which spores are produced singly rather than in chains; it is divided into two genera according to number of spores (Baldacci, 1959).

The genus Micromonospora (Oerskov, 1923) includes actinomycetes with short sporophores and with one spore on each. Bergey (1957) describes five species, and Krasil'nikov (1949)—nine species. M. chalcea (Foulerton, 1950) is the oldest species. The genus Microbispora (Nonomura et Ohara, 1957) includes actinomycetes with two spores (in pairs) on short sporophores. In the same year, microorganisms of the same group, such as Waksmania (Lechevalier et Lechevalier, 1957) and Thermopolyspora (Henssen, 1957) were described in Germany, but it seems to us that priority is on the side of the Japanese authors; aside from this, the name Microbispora is etymologically more indicative of the nature of these microorganisms. To date, only the species M. rosea (Kalakutskii, 1959) and Th. bispora (Henssen, 1957) have been described.

The family Actinomycetaceae (Buchanan, 1918) includes true actinomycetes which form spores in chains on aerial mycelium, i.e., on true sporophores. Since the majority of the actinomycete species described thus far belong to it, their classification is the most difficult of all. This family could have two genera distinguished by the structure of the ends of the sporophores.

The genus Actinomyces (Harz, 1877; emend. Teshich, 1959) includes actinomycetes with aerial mycelium and sporophores having straight ends, which reflects the meaning of the name itself (actis, actinis—ray-like, straight). There are 49 such species in Bergey, while in Krasil'nikov (1949), there are only 13 large species in which there are several dozen varieties with different forms of sporophore tips. A. odorifer (Rulmann, 1895) is the oldest species.

The genus Streptomyces (Waksman et Henrici, 1943; emend. Teshich, 1952) includes true actinomycetes with aerial mycelium; the ends of the sporophores are spiral, which is reflected in the name of the genus (streptus—bent, spiral). Bergey gives 48 such species while Krasil'nikov gives only 26. S. albus (Ross Doria, 1891) is the oldest.

This classification is based on the structure of the ends of the sporophores, and we agree with Okami's opinion (1952) that basically, there are two types of sporophores—straight and spiral (Shinobu, 1958). Other authors propose other groupings as well (Pridham et al., 1958; Ettlinger et al., 1958; Bisset, 1959) based on the general structure of the sporophores which can vary; however, as we have already shown their tips are always essentially of only two types. In some of the species indicated this character is not mentioned at all (Bergey and Krasil'nikov, for 13 species). For some species there are indications of both types existing together (Bergey for 12, and Krasil'nikov for 8 species), which must be checked on the specific species and varieties. If nutrient media of known composition (synthetic) are used in this case, more accurate data can be obtained (Krasil'nikov, 1949; Shinobu, 1958).

Finally, the family Actinoplanaceae (Couch, 1955) includes actinomycetes which form spores in sporangia. So far, two genera have been related to this family: Actinoplanes (Couch, 1950) with motile sporangiospores—species A. philippinensis (Couch, 1950), and Streptosporangium (Couch, 1955) with nonmotile sporangiospores—species S. roseum (Couch, 1955).

The rational solution of the problem of species in actinomycetes is the most difficult of all, but we believe that, if the existence of large biological species in nature is recognized, then it is possible to arrive at a definite rational division of these groupings into the genera mentioned. The division of old genera into two (even if they contain a large number of species) would already facilitate the solution of the problem. Many authors have suggested the unification of actinomycete species into "group species", sections, and series in conformance with botanical nomenclature. On the basis of certain morphological and physiological characters, Krasil'nikov (1949) formed ten such groups, Waksman (1959)—eleven, Kuster (1956)—ten, Hesseltine (1954)—seven. Baldacci (1959) first formed two sections according to the color of the substrate (primary vegetative) mycelium, and then divided them into 27 series by color of the aerial (secondary, sporogenous) mycelium. Pridham (1958) first formed seven morphological sections according to the morphology of the sporophores, and then divided them into six series on the basis of the color of mature spores and sporophores. It seems to us that Ettlinger (1958) did most of all along these lines when he grouped the species according to spore morphology (shape and surface), color of aerial mycelium (six main types), morphology of aerial mycelium (about 15 different types), and melanin production, and in this way brought together the previously existing 103 species into 34 larger species with a whole series of finer subdivisions. Twenty-six species gave no aerial mycelium, and probably formed it only on certain nutrient media. In our system, 22 of these 34 species belong to the genus Streptomyces and 12 to the genus Actinomyces. The second genus has smooth spores. Representatives of the first genus have spores with spines on the surface in the majority of cases.

Since our definitions of genera are based on the structure of the ends of the sporophores (straight or spiral), even greater opportunities arise for the demarcation of species on the basis of the entire structure and arrangement of the aerial mycelium and the sporophores on it (curved, various spirals, grouping of sporophores, etc.) and on the morphological characteristics of the spores (studied under the electron microscope). Other physiological and biochemical characters (relation to nutrient substances, enzyme systems, production of biotics and antibiotics, etc.) can also be considered for the further determination of strains, biotypes, and varieties within the bounds of the species in view of the fact that these, although they are variable characters, distinguish the individual strains in nature. In this case, properties appearing under the influence of ecological factors of the medium (aerobes, anaerobes, thermophilic, mesophilic, parasitic, saprophitic strains, etc.) would as well attain their full significance, since even Lehmann and Neumann (1912) regarded thermophilia as a property of the "race", while Kosmachev (1959) regarded it as a property of the species of these microorganisms.

SUMMARY

On the basis of all that has been said, we propose the following system for the more rational classification of actinomycetes, which differs somewhat from that proposed earlier (Teshich, 1959):

Class Actinomycetalia	—mycelium is formed or is absent
Subclass I. Mycobacteriineae	—mycelium absent in young cultures
Subclass II. Actinomycetineae	—mycelium formed in young cultures
Order I. Proactinomycetales	—septate mycelium
Family I. Proactinomycetaceae	—"spores" in chains
Genus I. Proactinomyces	—aerial hyphae are not formed
Genus II. Euactinomyces	—aerial hyphae formed
Order II. Actinomycetales	—nonseptate mycelium
Family II. Micromonosporaceae	—spores formed singly
Genus III. Micromonospora	—spores formed singly
Genus IV. Microbispora	—spores formed in pairs
Family III. Actinomycetaceae	—spores formed in chains
Genus V. Streptomyces	—sporophores with spiral ends
Genus VI. Actinomyces	—sporophores with straight ends
Family IV. Actinoplanaceae	—spores formed in sporangia
Genus VII. Actinoplanes	—sporangiospores motile
Genus VIII. Streptosporangium	—sporangiospores nonmotile

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THE AWARDING OF THE LENIN PRIZE FOR THE YEAR 1960 FOR INVESTIGATIONS IN THE FIELD OF MICROBIOLOGY

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The awarding of the annual Lenin prize for the most outstanding investigations in the field of science and technology is an important event in the life of our country.

Soviet microbiologists were very pleased to learn that, among the scientists honored by this high award in 1960, there are representatives of our science, namely, Professor Anatolii Evseevich Kriss.

The Lenin prize was awarded to Professor A. E. Kriss for the publication of the book, "Marine Microbiology (Deep-Sea)" in 1959. This book is the first and only monograph on oceanic microbiology.

In it are summarized the purposeful and original investigations of the author and his co-workers over a period of many years on the study of the quantitative distribution, species composition, multiplication rate and role of microorganisms in our Soviet seas and in the oceans of the world.

A. E. Kriss has made detailed studies of the microflora from the surface layer to the bottom of the Black, Caspian, Norwegian, Greenland, and Okhotsk Seas, and has studied the waters of the Arctic, Antarctic, Pacific, Atlantic, and Indian Oceans. In addition to water, he has examined the microflora of deep-sea sediments of many seas and oceans.

A. E. Kriss has frequently participated personally in expeditions on drifting North Pole stations, and was the first to study and describe the microflora of relatively inaccessible regions of the Arctic Ocean. Priority in the study of a number of uninvestigated regions of the oceans of the world and in the isolation of a number of unknown forms of microorganisms, among which were microorganisms with unusual morphology which he placed in a new class, rightly belongs to him.

The value of A. E. Kriss' monograph consists both of the fact that data are presented in it which are not based solely on the calculation and study of the quantitative and species composition of microorganisms capable of growing on artificial nutrient media, but also of the fact that he widely applied various methods of direct microscopy which enabled him to detect that microflora which does not grow on known nutrient media.

In A. E. Kriss' book, valuable information is given on the role of microorganisms in the biological productivity of the sea. He demonstrated the feasibility of using microorganisms as extremely delicate indicators of subsurface currents. The latter, as well as extensive information on the geographic distribution of microorganisms, makes A. E. Kriss's book, "Marine Microbiology (Deep-Sea)" a valuable text not only for microbiologists, but for hydrobiologists, hydrochemists, and oceanographers as well. This book is not only a very great biological, but also a great oceanographic investigation.

Convincing proof of the great value of A. E. Kriss' monograph is the fact that it is being translated in a number of countries: in England, Japan, China, and the German Democratic Republic.

A. A. Zubrilin and E.N. Mishustin, The Ensilage of Fodder (The Theory of the Problem). Popular Science Series, Izd. AN SSSR, Moscow, 1958. 225 pages. Price 4 rubles 50 kopecks.

Reviewed by E. V. Runov.

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In the solution of the problem of creating a fundamental feed basis for socialistic animal husbandry, the ensilage of feeds is of great significance as a simple and reliable method of storing the feed mass in a succulent state. This procedure for preparing feeds is widely employed in agriculture. It will be developed to an even greater extent in the coming years. According to the master figures for the development of the national economy of the USSR for 1959-1965, which were confirmed at the Eleventh Congress of the CPSU, at least a fourfold increase in the production of ensilage is anticipated. This task can be realized with maximal efficiency by applying the correct scientifically based ensilage technology which would ensure the production of high-quality silage from various types of raw material.

Thorough knowledge of the properties of ensilage serves as a reliable guarantee of its success. Such knowledge prevents mistakes in technology, eliminates spoilage, and makes it possible to act with greater assurance under production conditions. The book by A. A. Zubrilin and E. N. Mishustin will render a great service in this respect; the most important positions of the theory of ensilage are given in a very understandable way, with the generalization of advanced experiments and with practical conclusions.

The beginning of this book goes back to 1950, when "Ensilage", the work of A. A. Zubrilin, E. N. Mishustin, and V. A. Kharchenko, was published. The authors revised their previous material in accordance with the present state of knowledge concerning silage.

The book is distinguished by originality. Its content reflects both the authors' own rich experience and experimental results gathered by our domestic science over a long period of time.

The book opens with a brief introduction in which the essence and significance of ensilage are set forth. This is followed by a chapter on the conditions determining the ensilage process. They are regarded from the point of view of preventing loss in nutritional value of the feed on the basis of systematic analysis of the changes occurring in the feed.

The section on the isolation of feed from air pictures the life of cut plants, aerobic, and anaerobic respiration, death, the decomposition of carbohydrates and proteins, and the formation and evolution of carbon dioxide. The isolation of feed is apparently not the only condition for ensilage. Other factors as well—hydrogen ion concentration, temperature, moisture—must be considered in ensilage.

The section on the role of the acidification of feed acquaints one with electrolytic dissociation, the actual acidity, the buffered state and buffering capacity of plants.

In the next section, the significance of cutting up the fodder in the preparation of silage is clearly shown. It is correctly emphasized that quality is reduced if this condition is ignored. Cutting up the plant mass speeds the dying off of the plants, shortens the time of oxygen respiration of the cells, and promotes more rapid expression of juice and settling of the mass being ensilaged. Crushing promotes the initiation of lactic acid fermentation, and losses of nutrient substances from feeds are reduced.

Much attention is devoted to the effect of moisture from the point of view of controlling the microbiological process and its significance for the vital activities of microorganisms. Proceeding from theoretical premises, the principles of the preservation of feeds with reduced moisture are illuminated in detail, and the possibility of utilizing this method of ensilage in practice is explained.

In the section on the significance of temperature during ensilage, the two methods of ensilage—"hot" and "cold"—are discussed. A critical evaluation of hot ensilage shows it to be unprofitable. The advantages of the more profitable cold ensilage are seriously argued. The application of the hot method is confined to the limited role of ensilaging coarse feeds for the purpose of softening them.

The second chapter is devoted to the study of the sugar minimum, which is now recognized as a fundamental scientific basis for ensilage technology. A. A. Zubrilin deserves the credit for developing the study of the sugar minimum.

The sugar minimum theory is based on the development of lactic acid fermentation during ensilage on a scale where actual acidity adequate for the preservation of feed is attained in a short period of time. From this, the significance of sugar content in feed is characterized as a factor determining the course of ensilage. The reader is gradually led to understand the meaning of the sugar minimum and its final formulation as the percent of sugar necessary for the accumulation of lactic acid in ensilage in amounts ensuring a shift in the pH indicator of the silage to 4.2 at the given buffered state of the initial raw material. The sugar minimum index makes it possible to anticipate the quality of the silage ahead of time.

Methods are given for determining the sugar minimum, and for the classification of feeds according to the degree of their silageability, as well as calculations of the ratios of the silageable components of the raw material on the basis of the sugar minimum.

The reliability of the sugar minimum theory is confirmed by a number of data showing a direct relationship between silage quality and the sugar minimum.

A section on the supplementary buffering and acidification of feed introduces a refinement into the sugar minimum theory. Concrete examples are used to analyze fluctuations in time intervals required for the ripening of silage made from various cultures and containing adequate sugar, and the reasons for this phenomenon are determined. A detailed description is given of the rise of supplementary buffering in feeds caused by the accumulation of ammonia and the reduction of nitrates in the course of the ensilage process. The role of supplementary buffering in increasing the expenditure of sugar and in reducing the silageability of fodder is explained.

Aside from normal acidification of the silage, the question of the accumulation of excess acids in silage and measures for preventing this undesirable process are discussed.

The chapter ends with a section on procedures for promoting the silaging qualities of fodder. The rapid production of anaerobiosis, the utilization of the starch contained in the vegetable raw material, and the steaming of the fodder are accentuated.

As a whole, the chapters mentioned are well written. The materials are distinguished by substantiality. The essentials of ensilage and the variety of changes in fodder during the course of ensilage are revealed to the reader. At the same time, the reader gets a clear idea of the means for effectively influencing the course of the processes and of the possibility of applying precise and easily available methods of preserving any succulent feeds.

The next two chapters are devoted to the microbiology of ensilage. The first part acquaints one with the microflora of silage, while the second deals with the specifics of microbiological and enzymatic processes during ensilage. The account of the microorganisms inhabiting silage is prefaced by a brief outline of microscopic organisms, after which there is a description of the most important microbial groups, namely: lactic acid and butyric acid bacteria, bacteria of the colon group, acetic acid bacteria, cellulose-decomposing microorganisms, putrefactive bacteria, yeasts, and molds. The chapter also includes a section on the epiphytic microflora of plants.

The physiological and biochemical properties of microorganisms are given particular attention. Each individual group is treated from the point of view of its significance in ensilage. Lactic acid bacteria, to which the greatest part of the text is allotted, are given prominence. This is evidence of the observance of proportion and balance of the sections. Despite its brevity, the chapter gives a good characterization of the composition of silage microflora.

Data on microbiological processes in ensilaged fodder are set forth with strict logic and coherence. First, the main characteristics of the development of microbiological processes during ensilage are shown. Three phases in the fermentation of fodder are discussed—the phase of development of a mixed microflora, the phase of the basic lactic acid fermentation, and the final death phase of the microorganisms. Anomalies in the development of fermentative processes are clarified in passing.

Special sections are devoted to the dynamics of microbiological processes and acid production during cold ensilage of fodders of various chemical compositions. Some considerations are also offered concerning microbiological processes occurring during the hot method of ensilage.

The section on the heating of fodder is very instructive; the role of microorganisms in thermogenesis is rather fully developed in this section. It is demonstrated that thermogenesis is essentially an oxidative microbiological process.

The section on the activity of enzymes in the ensilaged mass is not closely tied in with microbiology. In our opinion, this should have been moved to the first chapters of the book, since enzymes are mentioned from the beginning of the account without the needed explanations.

The introduction to the microbiology of ensilage is completed by chapters concerning the utilization of lactic acid starters and preservatives in the ensilage of fodders. The chapter contains valuable confirmations of the expediency of using cultures of lactic acid bacteria for improving the ensilage process. The value of starters is illustrated by experimental data. Along with this, methods are given for preparing and applying bacterial silage starters.

In our opinion, the question of vitamins is discussed too briefly, despite the fact that the text points out the significance of vitamins as a most important factor in nutrition. The last chapter is devoted to the technology of ensilage. In it are discussed the following aspects: general procedures of harvesting and packing the green mass into the silo, methods of fodder ensilage depending on their silageability, the housing of silage, the ensilage of corn, silo buildings, terrestrial ensilage of fodders, losses during ensilage, and the determination of quality of feeds.

Let us go on to a discussion of some of the deficiencies of the book. In our opinion, the structure of the book is not entirely successful. Inasmuch as the elements of ensilage technology are closely interrelated with microbiology, it would have been useful to first familiarize the reader with the microflora of silage. Therefore, the section on the microorganisms of silage should have been placed at the beginning, after the introduction. It is also pertinent to mention that it is felt that, in the presentation of the materials, there is some lack of coherence between the technological and microbiological parts as shown by the repetitions encountered, which could have been avoided by a more unified structure of the book.

The style of writing and its simplicity are not everywhere maintained. The etymology of special foreign terms is not given.

In the section on the isolation of silage fodder from the access of air, the production of carbon dioxide in the silo by the heterotrophic group of lactic acid bacteria is not mentioned. The effect of the process of thermogenesis is the ensilage mass on the quality of the silage should have been more widely outlined, es

pecially in connection with the ensilage of fodders of sweet clover. In general, terrestrial ensilage, in view of its importance, requires more thorough discussion than is given in the book.

In the section on the determination of quality of fodders, the recommendation concerning the inadvisability of using plants which contain many alkaloids for silage is not accompanied by a list of such plants or even by a reference to the literature from which it would be possible to get the necessary information.

As a whole, the authors have successfully dealt with the task set before them—to illuminate in condensed form the principal questions of ensilage theory, and to demonstrate their practical significance and the conclusions resulting from them which are of interest to industry. The book written by them is characterized by purpose and by detailed disclosure of the most important interrelationships and patterns. It has every reason to become a handbook for a wide circle of workers in agriculture.

E. V. Runov

ORGANIZING CONVENTION OF THE ALL-UNION MICROBIOLOGICAL SOCIETY

The vigorous development of microbiology and its incorporation into the most varied areas of the national economy has introduced new problems in the field of the organization of microbiological science and has strengthened its connection with industry. An important measure in this direction was the decision of the Presidium of the Academy of Sciences, USSR on the creation of the All-Union Microbiological Society. The greatest Soviet microbiologists were included in the Organizing Committee; Corresponding Member of the Academy of Sciences, USSR A. A. Imshenetskii was installed as its Chairman. The Organizing Committee has conducted a great deal of work on the organization of the divisions of the Society and on preparations for its final organizational formation.

The First (Organizing) Convention of the All-Union Microbiological Society was held in Moscow, January 19 and 20, 1960. Ninety-nine delegates and 43 invited guests were present at the convention. The delegates to the convention represented 12 divisions of the Society which at present number more than 1000 members and are organized in the Armenian, Belorussian, Kazakh, Latvian, Lithuanian, Uzbek, Ukrainian, and Georgian Union Republics, as well as in the cites of Moscow, Leningrad, Novosibirsk, and Kazan.

The convention was opened by Acting Academician-Secretary of the Division of Biological Sciences of the Academy of Sciences, USSR Academician N. M. Sisakyan, who in a brief introduction noted that in the historic resolutions of the Eleventh Congress of the Party, microbiology and virology were included among the most important disciplines which are destined to develop rapidly, pointed out the scientific and practical significance of microbiological investigations, and emphasized the importance of the organization of the Microbiological Society.

The Chairman of the Organizing Committee of the Society, Corresponding Member of the Academy of Sciences, USSR A. A. Imshenetskii, presented a long report on the tasks of the Society in the light of the resolutions of the Eleventh Congress of the CPSU and the subsequent plenums of the Central Committee of the CPSU. The speaker directed particular attention to the fact that, along with the scientific activity and work on the coordination of investigations, one of the central problems of the Society is the establishment of a close connection between microbiological scientific research institutions and industrial organizations. The Society must in every way expedite the working out of problems important to the national economy, the incorporation of microbiological achievements into industry, and the raising of the scientific qualifications of industrial workers. In accordance with this, the planned regulations of the Society provide for the extensive inclusion of industrial workers in the ranks of the Society.

Representatives of the State Planning Committee of the Council of Ministers, USSR, Candidate of Technical Sciences V. L. Yarovenko, of the Ministry of Public Health, USSR, Candidate of Technical Sciences A. G. Natradze, and of the All-Union Academy of Agricultural Sciences, Candidate of Biological Sciences Ya. P. Khudyakov made appearances at the convention. They pointed out a number of problems which are at present facing industry and agriculture and in the solution of which microbiological scientific research establishments and the Microbiological Society must assist. Among the most important of these questions is the production of new active strains of microorganisms which form biologically active substances (antibiotics, vitamins, enzymes, growth stimulants, etc.) and the further improvement of the technology of producing the latter; the development of microbiological methods of the transformation of steroids, which requires wide expansion of investigations in this field; the microbiological production of alkaloids; the production of feed antibiotics; the search for new cultures of microorganisms which would make it possible to utilize the raw material of the fermentation industry more completely (pentoses, for example); the development of microbiological methods for increasing the fertility of soils (particularly the study of the optimal combinations of organic, mineral, and bacterial fertilizers, as well as conditions for the development of a useful soil microflora); the ensilage of fodders, etc.

The representative of the All-Union Society of Epidemiologists, Microbiologists, and Sanitarians, Active Member of the Academy of Medical Sciences, USSR V. D. Timakov, read a long paper; he emphasized the importance of close association between the Society of Epidemiologists, Microbiologists, and Sanitarians and the newly created All-Union Microbiological Society. The speaker pointed out a number of problems the solution of which requires particularly intensive cooperative work. These included: heredity and variability in microorganisms, the mechanism of action of antibiotics and the production of microbial forms resistant to them, problems of general virology and bacteriophagy, particularly the physiology of viruses, their reproduction and means of dissemination and survival in nature.

The Convention heard the report of the Organizing Committee of the Society and reports by representatives of the councils of its divisions, who explained the work done on the organization of the divisions, their activity, and the problems confronting them in connection with local conditions of development of microbiology.

Finally, the Convention approved the code of the Society, elected the executive body, and adopted a resolution defining the basic problems of current work.

In the code of the Society and in the resolution adopted by the Convention, it is emphasized that the basic task of the Society is to unite scientific and industrial

workers for the development of microbiological and virological investigations as the basis of intensifying assistance to industry, agriculture, and public health.

It is also noted in the resolution that the Society must take an active part in the planning and coordination of work important for the national economy; it must organize scientific information by means of publishing a periodical organ of the Society, the transactions of the Society, monographs, and brochures; it must improve the training and raise the qualifications of cadres of microbiologists; it must promote the extention of international ties by active participation in the sending of delegations to international scientific conferences and symposia on microbiology and virology as well as allied disciplines (biochemistry, botany, genetics, soil science, etc.).

The Convention elected a Central Council of the Society, which is to include the directors of its divisions in the Union Republics and large cities as well as the most prominent scientists representing the most important branches of microbiology (a total of 51 people).

The Central Council elected the Director of the Institute of Microbiology of the Academy of Sciences, USSR and Corresponding Member of the Academy of Sciences, USSR A. A. Imshenetskii as President of the Society, Corresponding Member of the Academy of Sciences of the Armenian SSR A. K. Panosyan and Prof. I. Ya. Veselov as Vice Presidents, Candidate of Biological Sciences A. B. Lozinov as Academic Secretary, and Academician V. N. Shaposhnikov, Corresponding Member of the Academy of Sciences, USSR A. N. Krasil'nikov, Academician of the Academy of Sciences of the Ukrainian SSR V. G. Drobot'ko, Active Member of the Academy of Medical Sciences, USSR V. D. Timakov, Professors N. D. Ierusalimskii, V. I. Poltev, Ya. I. Rautenshtein, M. N. Rotmistrov, B. Ya. El'bert, and Candidates of Biological Sciences K. I. Brundz and Ya. P. Khudyakov as Members of the Presidium.

The Convention also elected an Examining Commission, the task of which includes the control of all aspects of activity of the Society (scientific, organizational, and financial). The Examining Commission is composed of: Doctor of Biological Sciences V. I. Kudryavtsev (Chairman), Corresponding Member of the Academy of Sciences, USSR E. N. Mishustin, Doctor of Biological Sciences I. L. Rabotnova, Candidates of Biological Sciences É. V. Afrikyan, V. D. Kuznetsov (Secretary), M. M. Makarova, G. K. Skryabin.

A. B. Lozinov

EXTENDED MEETING OF THE SCIENTIFIC COUNCIL ON THE PROBLEM "CONTROL OF THE METABOLISM OF MICROORGANISMS FOR THE PURPOSE OF REGULATING MICROBIOLOGICAL PROCESSES IN INDUSTRY AND AGRICULTURE"

On January 25, 1960, the Institute of Microbiology of the Academy of Sciences, USSR held an extended meeting of the Scientific Council on the problem, "Control of the metabolism of microorganisms for the purpose of regulating microbiological processes in industry and agriculture". Members of the Scientific Council on this problem, representatives of the Academies of Sciences of the Union Republics, of branch scientific research institutes and directors of departments of microbiology, etc., were present at the meeting.

A communication from the Chairman of the Scientific Council, Corresponding Member of the Academy of Sciences, USSR A. A. Imshenetskii was heard concerning the basic direction of the work regarding this problem for the next 15 years.

A. A. Imshenetskii noted that in becoming acquainted with the scientific plans of the Academies of Sciences of the Union Republics and the affiliated and branch scientific research establishments, it was determined that the scientific establishments are still engaging very little in the study of questions of the physiology and biochemistry of microorganisms and are not employing new methods of investigation in their work (especially in soil microbiology).

A. A. Imshenetskii expressed the hope that, in making scientific plans, the opportunities and requirements of the particular republic, region, and district will be taken into account, and that investigations conducted at the central scientific research establishments not be duplicated.

Following the paper, there was a wide exchange of opinions.

For the purpose of strengthening the work on the physiology and biochemistry of microorganisms, the speakers noted the extreme necessity for creating nuclei of microbiologists in the USSR who would be called upon to work out certain aspects of the problem on a high theoretical and methodological level, using physiological methods of investigation and the newest techniques. Along with this, it was thought to be expedient to carry on wide exchange of experience (through print, scientific conferences, meetings, etc.) concerning work on the various questions of microbial physiology.

As the result of discussing the new content of the problem, the Convention felt it expedient to retain in it only those sections which have a direct relationship to the study of the metabolism of microorganisms.

Proceeding from this, it was decided that for the next 15 years, scientific investigations of the problem must be oriented toward the following:

I. First of all, the study of the physiology of metabolism of various microorganisms in connection with the conditions of their growth, intensifying investigations of poorly known microflora occurring in nature (in soil, silts, bodies of water, etc.).

II. The determination of laws of experimental variability caused by various physical and chemical factors in connection with the metabolism of microorganisms.

III. The development of scientific bases for the rationalization of existing industries and the organization of new ones, particularly those connected with obtaining fermentation products and physiologically active substances (antibiotics for medicine and agriculture, growth stimulants for animals and plants, vitamins, enzymes), as well as with the use of microorganisms

to carry out particular reactions in the synthesis of certain steroid hormones.

IV. The intensification of studies of the biochemical activity of microorganisms carrying out the decomposition and synthesis of humus, the nitrogen cycle, and other conversions in the soil, and working out a system of measures for mobilizing the latent resources of soil fertility.

V. The deepening and expansion of physiological investigations of microorganisms occurring in nature, especially of microorganisms in ore deposits. In connection with this, it is assumed that recommendations for regulating their activity will be given.

In order to insure the successful performance of the investigations outlined, it was considered essential to intensify the training of scientific cadres of microbiologists. For this purpose, it is expedient to restore departments of microbiology in those universities where they have been abolished for one reason or another. Along with the training by universities of microbiologists with a physiological orientation, it would be desirable to attract specialists with good knowledge of biochemistry and organic chemistry to postgraduate study in microbiology. It is also necessary to take measures to raise the qualifications of scientific co-worker-microbiologists in the fields of physical and chemical methods of investigation. In addition to this, the cadres of microbiologists should be considerably increased in a number of branch institutes, primarily in the Vitamin and Chemicopharmaceutical Institutes and in the Institute of Animal Husbandry and Feeds, the Institute of Grain, etc. It is necessary to expand the network of agronomic laboratories at branch scientific research institutes in the VASKhNIL system (All-Union Academy of Agricultural Sciences), as well as to create departments of microbiology in agricultural institutions of higher learning.

The extended convention which was held directed the aims of the nation's microbiologists toward the solution of the most important problems facing modern microbiology—the study of the metabolism of principally those microorganisms which are of important scientific-theoretical or practical significance.

I. G. Kolesnikova

PROBLEMS IN THE MICROBIOLOGY OF FEEDS

In December of last year, a convention was held in Alma-Ata on the microbiology of feeds, which was called in accordance with the plan of the Council of the Coordination of the Academy of Sciences, USSR for the year 1959.

The Biological Division and the Institute of Microbiology of the Academy of Sciences, USSR, the Kazakh Division of the All-Union Microbiological Society, the Institutes of Agricultural Microbiology, Animal Husbandry, and Feeds of the All-Union Institute of Agricultural Sciences, the Division of Biological and Medical Sciences of the Academy of Sciences of the Kazakh SSR, the Institutes of Microbiology, Virology, and Physiology of the Academy of Sciences of the Kazakh SSR, the Institute of Feeds of the Kazakh Academy of Agricultural Sciences, and the Moscow Division of the All-Union Institute of the Hydrolysis and Sulfite-Alcohol Industry took part in the work of the convention.

Academician-Secretary of the Division of Biological and Medical Sciences of the Academy of Sciences of the Kazakh SSR N. U. Bazanova opened the convention with a few introductory words.

The Director of the Institute of Microbiology and Virology of the Academy of Sciences of the Kazakh SSR D. L. Shamis presented a paper concerning work on the microbiology of feeds in Kazakhstan.

S. Ya. Zafren (Institute of Feeds of the All-Union Academy of Agricultural Sciences) reported on the significance of the antimicrobial properties of raw material during the ensilage of fodders. Here, the same factors are utilized which aid live plants to withstand attacks by microorganisms. The investigation of the natural antimicrobial properties of silageable raw material makes it possible to further work out the most rational methods of preserving fodder.

Corresponding Member of the Academy of Sciences, USSR E. N. Mishustin (Institute of Microbiology of the Academy of Sciences, USSR) devoted his paper to the role of the science of microbiology in solving the feed problem. In speaking of the immediate problems of the theory and practice of the ensilage of fodder, the speaker dwelt in detail on the characteristics of terrestrial ensilage. This procedure is economically profitable and more easily solves the organizational problem of feeding cattle with silage. However, the possibilities of losses increase considerably during terrestial ensilage. The principal reasons for losses were thoroughly discussed in the paper and recommendations for eliminating them were given. Particular attention was given to the phenomenon of spontaneous heating of ensilage fodder leading to the conversion of valuable nitrogen-containing components of the fodder to compounds which cannot be assimilated by animals. The speaker devoted a considerable portion of his presentation to measures of combatting thermogenesis of ensilage.

M. M. Makarova (Institute of Agricultural Microbiology of the All-Union Academy of Agricultural Sciences) reported to the convention on the biological methods of controlling ensilage processes and on the future possibilities of their utilization. It was shown that the use of starters of pure cultures of lactic acid bacteria promotes better preservation of proteins, soluble carbohydrates, and vitamins in the feed; it decreases waste of the feed in the surface layers by 1.5–3 times. In order to improve the quality of silage and to reduce waste during terrestrial ensilage, it is necessary to broaden investigations and to speed up the development of a factory method for preparing a combination starter of lactic acid bacteria and yeast.

M. S. Karpov (Institute of Microbiology and Virology of the Academy of Sciences of the Kazakh SSR) gave a paper on the utilization of straw in the ensilage of fodder.

K. K. Kurmanov (Institute of Feeds of the Kazakh Academy of Agricultural Sciences) illuminated certain

questions of the ensilage of feeds in Kazakhstan. Corn—the most important silage culture—occupies an important place in the republic; the growth of areas under plantings is due to the utilization of virgin and waste lands. Corn plantings in the northern regions account for about 60% of the total area of corn plantings in Kazakhstan.

The participants of the convention noted that, in the field of terrestrial ensilage, practice is far ahead of science. This obliges scientific workers to push ahead on the technology of preparing silage by the terrestrial method. In this connection, the question requiring the most rapid solution is that of thermogenesis which brings serious losses during ensilage. Disregard of temperature conditions during the packing of silage leads to low yields of it (E. N. Mishustin, S. Ya. Zafren, and others).

A certain amount of attention at the convention was devoted to the utilization of fodder antibiotics in animal husbandry. V. A. Alekseev (Institute of Agricultural Microbiology of the All-Union Academy of Agricultural Sciences) reported on a simplified method of preparation and the results of the application of terramycin in swine husbandry. Yu. M. Gononov and N. A. Glebova (Institute of Veterinary Scientific Research of the Kazakh Academy of Agricultural Sciences) reported to the convention on the effectiveness of the application of antibiotics on poultry-raising farms in the Alma-Ata region. They showed that antibiotics, especially biomycin and synthomycin, are exceptionally active measures in the prevention of chick diseases.

Part of the papers were devoted to questions pertaining to the effect of ensilage fodder on the secretion of gastric juice (M. K. Stepankina) and the use of acidophilic preparations in animal husbandry (V. V. Leonovich and M. S. Polonskaya). N. S. Nikitina (All-Union Scientific Research Institute of the Hydrolysis and Sulfite-Alcohol Industry) reported on the results of work concerning the enrichment of straw with protein on the basis of its fermentative treatment.

The question of the nature of bacterial starters drew a lively discussion. M. M. Makarova spoke for the preparation of liquid starters. She feels that nonsporogenous bacteria show considerable death of cells in dry starters. E. N. Mishustin, Ya. I. Rautenshtein, and others do not agree with the orientation in favor of liquid starters, inasmuch as dry preparations are considerably more transportable and convenient in application.

The utilization of virgin lands in Kazakhstan has made the problem of the utilization of straw of prime importance. The participants of the convention arrived at the unanimous opinion that work with this material is of cardinal significance.

In their presentations, the participants of the convention spoke of the great effectiveness of using antibiotics in the practice of animal husbandry. At present, it is still unquestionably too early to speak of the utilization of pure antibiotic preparations, but neither should one go to the other extreme, i.e., suggest homemade methods of preparing feed antibiotics. Feed antibiotics, as in the case of other biological preparations, must definitely pass government control (Ya. I. Rautenshtein, V. A. Alekseev, and others).

The convention believes that investigations in the field of the microbiology of feeds must be of a complex character with the most extensive participation of microbiologists, zootechnologists, and other specialists in the agricultural industry. The coordination of scientific research work which is in existence at the present time is entirely inadequate. The elimination of this and other shortcomings will enable scientific workers to contribute to the utmost of their abilities to the solution of the problem of feeds for animal husbandry.

Sh. A. Chulakov

DEFENSE OF DISSERTATIONS

I

On February 16, 1960 at the Kiev State University, I. I. Shevtsova presented her thesis for the degree of Candidate of Biological Sciences on the subject: Changes in the Properties of Some Soil Bacteria in the Rhizosphere of Agricultural Plants.

A study of the morphology, physiology, acclimatization, and condition of phosphorus and silica bacteria in the rhizosphere of corn, wheat, clover, and lucerne showed that these plants exert an influence on the quantitative and qualitative composition of the rhizosphere microflora in the course of their vital activities. Thus, the conditions of the root systems of wheat and lucerne promote the multiplication of phosphorus bacteria; silica bacteria grow best of all in the rhizosphere of corn. The largest number of phosphorus and silica bacteria under corn was observed during the blossoming period, under wheat during the periods of blossoming and milky ripeness, while in the rhizosphere of clover and lucerne, there was a maximum number of phosphorus and silica bacteria during the budding and blossoming stages.

The plants caused changes in the morphological and cultural properties of bacteria growing in their rhizospheres. In phosphorus bacteria, there were changes in cell dimensions, colony form, character of pigmentation, behavior toward certain carbon sources, and activity of mineralization of organic phosphates. In silica bacteria, colony form, size of capsules, elasticity of the slime, behavior toward certain sugars and nitrogen sources, and extent of alumosilicate decomposition underwent changes.

Experiments with root-nodule bacteria showed that root secretions as well as cellular juice and root tissues of plants can cause changes in the properties of these bacteria. In this case, root-nodule bacteria underwent changes in morphological and biochemical properties, as well as in degree of virulence, through the effect of tissue juices from the roots of clover and lucerne. It was shown that the variability of the bacteria studied was different in the rhizospheres of various plants and depended on the species and age of the plants, while the character of the effect of root

tissue juices on root nodule bacteria also depends on the concentration of the root juice.

The undiluted slurry of root tissue of the leguminous plant always had a bactericidal effect on root nodule bacteria regardless of whether or not the root nodule bacteria used were specific for the given plant. The extent of bactericidal action of the root tissues did not remain constant, but changed with the growth of the plant. Tissue juice from the roots of young plants proved to be the most bactericidal.

Later, the degree of bactericidal activity of the root tissues of the leguminous plants decreased somewhat, while the root tissues of clover and lucerne in the blossoming stage were found to be the least bactericidal. As the result of prolonged growth of the bacteria in the rhizosphere of a particular plant, strains of phosphorus and silica bacteria adapted to the conditions of the rhizosphere of the given plant were obtained. When these bacteria were used for the bacterization of seeds they became better acclimatized to the root system of the corresponding plant than did the original bacteria. At the same time, they formed smaller numbers of spores and remained in the active stage longer.

In some of the strains of bacteria tested, the beneficial properties acquired (better acclimatization in plant rhizospheres, stronger biochemical activity) became fixed upon further growth in the zone of the root system of the same plants, while in other strains, they were lost.

In greenhouse and field experiments, adapted strains promoted larger increases in the crops of the corresponding plants than did the original strain in the majority of cases.

II

On March 14, 1960, at the meeting of the United Scientific Council of the Division of Biological Sciences of the Academy of Sciences of the Uzbek SSR in Tashkent, P. T. Malakhova presented her thesis for the degree of Candidate of Biological Sciences on the subject: The Rhizosphere Microflora of Lucerne on Typical Irrigated Sierozem.

It was established that the rhizosphere microflora of lucerne is characterized by a specific composition of microorganisms. The number of the latter changes according to the developmental phases of the plant, reaching a maximum in the root and near-root zones during the periods of budding and blossoming.

It was found that the selective action of lucerne roots is manifested principally with regard to nonsporogenous bacteria (denitrifiers, bacteria growing on bean, sucrose, Ashby, root, and soil agars). Spore-bearing ammonifiers and azotobacter are not selected by lucerne.

The occurrence of bacteria around roots of different ages was found to be unequal. On young, active roots, certain bacteria were observed to be present in amounts tens of times larger than on old roots. Sporogenous and cellulose-decomposing bacteria, on the contrary, accumulated in large numbers on old, somewhat suberized roots.

On the roots and in the soil surrounding the roots of lucerne, 32 different species of bacteria were found, of which eight belonged to the genus Bacterium, three to Chromobacterium, 14 to Pseudomonas, three to Rhizobium, two to Pseudobacterium, and two to Micrococcus.

It was shown that under various growing conditions (typical sierozem and light saline sierozem), lucerne forms rhizosphere complexes which are similar not only in genus, but in species composition as well. However, these complexes are not identically distributed in the individual zones.

It was established that many of the rhizosphere bacteria of lucerne dissolve poorly soluble mineral and organic phosphorus compounds (tricalcium phosphate and nucleic acid). Bacteria of the genus Chromabacterium were found to be the most active in this respect.

The majority of nonsporogenous bacteria of lucerne rhizosphere grow well during mixed growth on certain nutrient media. Antagonism was observed only among a few species, and more frequently between sporogenous bacteria on the one hand, and between nonsporogenous ones on the other.

It was shown that the rhizosphere bacteria of lucerne have a significant effect on the germination of its seeds. Under laboratory conditions, 45% of the rhizosphere bacteria tested stimulated the germination of lucerne seeds, 14% inhibited this process, 10% retarded it, and 31% had no effect. Of the bacteria which stimulated the germination of seeds, only 16% of the strains tested had a favorable effect on the subsequent growth of the plant. Chromobacterium denitrificans strain 73 and Rhizobium meliloti strain 142 were found to be the most effective of these.

It was established that treatment of seeds with these bacteria under conditions of small-plot field experiments promoted greater production of nodules on the roots of the plant and increased the yield of the harvested mass by 4–6.7 centners/hectare.

III

On April 11, 1960, at Kiev State University, V. A. Kordyum presented his thesis for the degree of Candidate of Biological Sciences on the subject: Interrelationships of Nitrogen-Fixing Bacteria and Phosphorus Bacteria.

A study of natural and artificially caused variability in nitrogen-fixing bacteria and in phosphorus bacteria showed that their morphological characters are easily changed through the action of growth conditions and other factors.

It was established that when nitrogen-fixing bacteria and phosphorus bacteria were grown jointly on Ashby's liquid and agar media, despite an over-all positive effect, there were elements of bilateral antagonism.

The variability of the interrelationships between nitrogen-fixing bacteria and phosphorus bacteria depended on differences in phosphorus bacteria variants. As the result of selection, 26 variants of phosphorus bacteria were obtained, of which some stimulated the

growth of nitrogen-fixing bacteria while others inhibited it.

In addition, there were a number of cultures which were intermediate in their behavior toward nitrogen-fixing bacteria. The interrelationships of the phosphorus bacteria variants obtained with nitrogen-fixing bacteria were studied in detail under various growth conditions. When the results obtained were compared, it was found that the character of the relationships between nitrogen-fixing bacteria and phosphorus bacteria depends not only on the variant of the latter, but also on the composition of the nutrient medium and on culture conditions. The solid phase played a particularly important role during submerged cultivation of the cultures.

The feasibility of producing bacterial fertilizers by the continuous method was studied. Positive results were obtained.

It was established that, aside from influencing the morphologic-cytological and cultural characters, mixed cultivation of nitrogen-fixing bacteria and phosphorus bacteria leads to the alteration of the nitrogen-fixing and phosphate-mineralizing capacity of the association. Some variants of phosphorus bacteria intensify nitrogen fixation, others weaken it, while still others have essentially no effect on it.

While exhibiting various effects on nitrogen-fixing bacteria different variants of phosphorus bacteria themselves were unequally affected by it. This was reflected both in their morphologic-cytological characters and in their phosphate-mineralizing capacity.

It was established that the majority of phosphorus bacteria variants prevent the passage of nitrogen-fixing bacteria to the roots of barley and corn; some variants grow less well in the rhizosphere in the presence of Azotobacter chroococcum, and only a few stimulate the overgrowth of the roots by nitrogen-fixing bacteria and themselves grow better on the roots in its presence.

Aside from the effect on each other, the bacteria studied also had a certain effect on the plants. It was found that many variants of phosphorus bacteria can, under certain conditions, compete with the plant for nutritional elements. The addition of nitrogen-fixing bacteria in such cases leads to a negative result if it increases the growth of Bacillus megaterium var. phosphaticum in the rhizosphere. However, some variants of phosphorus bacteria do not compete with the plant, and then the addition of nitrogen-fixing bacteria promotes better growth of the plant.

Micro-plot experiments with corn showed that unstable and stable variants of phosphorus bacteria play a decisive role in the character of the effect on the plant and crop increase. Treatment of the seeds with the stable strain 79 of phosphorus bacteria and with nitrogen-fixing bacteria led to a 49% increase in the yield of corn. However, bacterization with a complex preparation consisting of the original heterogenic strain of phosphorus bacteria and A. chroococcum strain "K" did not give an increase in yield as compared with the control.

A. E. Kosmachev

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

AIBS Russian Monograph Translations

The AIBS is in the process of expanding its Russian Translations Program extensively. Funds to subsidize translation and publication of important Russian literature in biology have been obtained from the National Science Foundation, as part of a larger program to encourage the exchange of scientific information between the two countries. The following monographs have been published:

Origins of Angiospermous Plants. *By A. L. Takhtajan.*
Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin.
68 pp. $3.00, individuals and industrial libraries (U.S.A. and Canada);
$2.00, AIBS members and all other libraries; $.50, additional, foreign.

Essays on the Evolutionary Morphology of Plants. *By A. L. Takhtajan.*
Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin.
144 pp. $5.00, individuals and industrial libraries (U.S.A. and Canada);
$4.00, AIBS members and all other libraries; $.50, additional, foreign.

Problems in the Classification of Antagonists of Actinomycetes. *By G. F. Gause.*
Edited by David Gottlieb. Translated by Fritz Danga.
$5.00, individuals and industrial libraries (U.S.A. and Canada);
$4.00, AIBS members and all other libraries; $.50, additional, foreign.

Arachnida. Vol. IV, No. 2. Fauna of the U.S.S.R. *By B. I. Pomerantzev.*
Edited by George Anastos. Translated by Alena Elbl.
$10.00, individuals and industrial libraries (U.S.A. and Canada);
$9.00, AIBS members and all other libraries; $1.00, additional, foreign.

Arachnoidea. Vol. VI, No. 1. Fauna of the U.S.S.R. *By A. A. Zachvatkin.*
Translated and edited by A. Ratcliffe and A. M. Hughes.
$10.00, individuals and industrial libraries (U.S.A. and Canada);
$9.00, AIBS members and all other libraries; $1.00, additional, foreign.

Marine Biology. Vol. XX, Trudy Institute of Oceanology. *Edited by B. N. Nikitin.*
$10.00, individuals and industrial libraries (U.S.A. and Canada);
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DOKLADY

BIOLOGICAL SCIENCES SECTIONS

6 issues per year. First issues published: 1957, Vols. 112-117. Current issues published: 1960, Vols. 130-135.

This is a translation of all papers in the Biological Sciences Sections of the Proceedings of the Academy of Sciences of the USSR. It includes sections in many areas of biology, among which are the following: biophysics, cytology, embryology, experimental morphology, genetics, histology, hydrobiology, microbiology, morphology, physiology, and zoology.

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This journal is a translation of all papers in the Botanical Sciences Sections of the Proceedings of the Academy of Sciences of the USSR. It includes sections on botany, plant anatomy, plant embryology, plant morphology, and plant physiology.

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(FIZIOLOGIYA RASTENII)

6 issues per year. First issues published: 1957, Vol. 4, Nos. 1-6. Current issues published: 1960, Vol. 7, Nos. 1-6.

This journal publishes original studies of an experimental nature on various problems of the physiology of plants, reviews, critical articles, and discussions of major problems in this field. It has special sections dealing with methods of experimental research and the application of finished scientific research to current agricultural practice.

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(MIKROBIOLOGIYA)

6 issues per year. First issues published: 1957, Vol. 26, Nos. 1-6. Current issues published: 1960, Vol. 29, Nos. 1-6.

This journal deals with problems of general microbiology (systematics, morphology, physiology, and variability of microorganisms), agricultural microbiology (microbiology of soils and fodder crops, bacterial fertilizers), technical microbiology, the microbiology of foods, and the microbiology of water reservoirs. This journal also contains sections on the history of science, book reviews, and current events in the field of microbiology, as well as the general articles in this field.

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ENTOMOLOGICAL REVIEW

(ENTOMOLOGICHESKOE OBOZRENIE)

4 issues per year. First issues published: 1958, Vol. 37, Nos. 1-4. Current issues published: 1960, Vol. 39, Nos. 1-4..

Very few entomologists would not find something of special interest to them in any one issue of this periodical. It deals with theoretical and practical problems in the field of entomology and their significance for the national economy. It contains articles on all branches of entomology and devotes particular attention to problems connected with the theoretical basis of agriculture and public health service.

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(POCHVOVEDENIYE)

12 issues per year. First issues published: 1958. Current issues published: Jan.-Dec. 1959.

This is a translation of the Russian journal, Pochvovedeniye, edited by Dr. A.P. Mazurak of the University of Nebraska. It publishes research done at various institutes in the fields of soil chemistry, soil physics, soil microbiology, soil fertility, soil genesis and classification, soil conservation, irrigation, and related aspects of soil science.

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